MICROBIAL DECOMPOSITION OF PINE (*Pinus kesiya*) LITTER : AN ECOLOGICAL STUDY

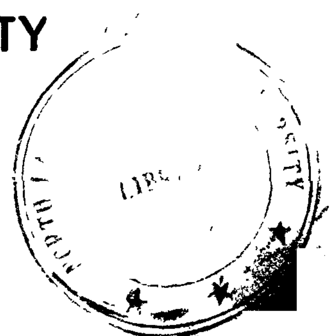
BY

PIJUSH KANTI DAS, M.Sc.
DEPARTMENT OF BOTANY

THESIS SUBMITTED IN FULFILMENT OF THE
REQUIREMENT OF THE DEGREE OF
DOCTOR OF PHILOSOPHY IN BOTANY

TO

THE NORTH EASTERN HILL UNIVERSITY
SHILLONG (INDIA)
1980



DS
585.2045
DAS

Released

NEHU Library
Acc. No. 58352
Acc. by
Class by
Sub. Heading by
Cat.
Transcribed by

SUPERVISOR'S CERTIFICATE

I certify that the thesis entitled "Microbial Decomposition of Pine (Pinus kesiya) Litter : An Ecological Study" submitted by Mr. Pijush Kanti Das for the Degree of Doctor of Philosophy of the North-Eastern Hill University, Shillong embodies the record of original investigation carried out by him under my supervision. He has been duly registered and the thesis presented is worthy of being considered for the Award of the Ph.D. Degree. This work has not been submitted for any Degree of any other University.

Date 7.2.1980

Place Shillong

R.R. Mishra

Signature of the Supervisor

R. R. Mishra
Professor of Botany
School of Life Sciences
NEHU, Shillong-793003, India

ACKNOWLEDGEMENTS

I wish to express my deep sense of gratitude to Dr. R.R.Mishra, Professor, Department of Botany, North-Eastern Hill University, Shillong for his able guidance, valuable suggestions and constant encouragement throughout the course of present investigation.

I also acknowledge indebtness to Professor P.S. Ramakrishnan, Head of the Department of Botany, North-Eastern Hill University for his keen interest in the present study and providing laboratory facilities.

I am obliged to U.G.C. for providing the grant to carry out the present investigation.

Shillong
Dated 7.2.1980

Pijush Kanti Das
(Pijush Kanti Das)

CONTENTS Page Nos.

GENERAL INTRODUCTION 1

CHAPTER I - SEASONAL VARIATION IN THE POPULATION AND ACTIVITY OF THE SOIL MICROORGANISMS IN THE PINE FOREST

INTRODUCTION 6

REVIEW OF LITERATURE 12

MATERIALS AND METHODS 23

RESULTS 35

DISCUSSION 49

CHAPTER II -ECOLOGY OF LEAF SURFACE MICROORGANISMS OF PINE NEEDLES

INTRODUCTION 59

REVIEW OF LITERATURE 62

MATERIALS AND METHODS 70

RESULTS 76

DISCUSSION 86

CHAPTER III - MICROBIAL DECOMPOSITION OF PINE LEAF LITTER

INTRODUCTION 94

REVIEW OF LITERATURE 98

MATERIALS AND METHODS 109

RESULTS 124

DISCUSSION 147

CONTENTS Page Nos.

CHAPTER IV - EFFECT OF THE AMENDMENT OF PINE LEAF LITTER ON SOIL MICROBIAL POPULATION

INTRODUCTION	160
REVIEW OF LITERATURE	163
MATERIALS AND METHODS	166
RESULTS	170
DISCUSSION	179
GENERAL DISCUSSION	183
ABSTRACT	190
BIBLIOGRAPHY	195

GENERAL INTRODUCTION

Decomposition of litter is one of the most important phenomenon in the ecosystems. The biological degradation of the plant litter represents one of the essential links in the natural process of carbon circulation (Vladimir, 1970).

The role of microorganisms in the decomposition of plant material is of immense importance. The microbes are primarily responsible for the release of various nutrients locked up in the plant tissue which in turn are utilized by other organisms. The cycling of minerals in an environment is to a great extent influenced by the microbial activities of the region. Obviously, any influence in the normal functioning of the microbes during decomposition processes will effect the cycling of the minerals.

The increased interest in the role of microorganisms in nutrient and energy flow relationships in natural as well as man-manipulated environments has emphasized the need of litter decomposition studies. In the process of litter decomposition microbial attack is the most important and the process becomes further complicated since large variety of microorganisms are involved. To understand this complex phenomenon of decomposition of plant litter it is logically important to evaluate the role of the most important groups of organisms, their succession and their natural influence.

In north-eastern part of the country where the climatic fluctuations are extreme, the studies relating to role of microorganisms in the decomposition of litter in the forest, release of nutrients and phytotoxins during decomposition and the effect of latter on soil microflora

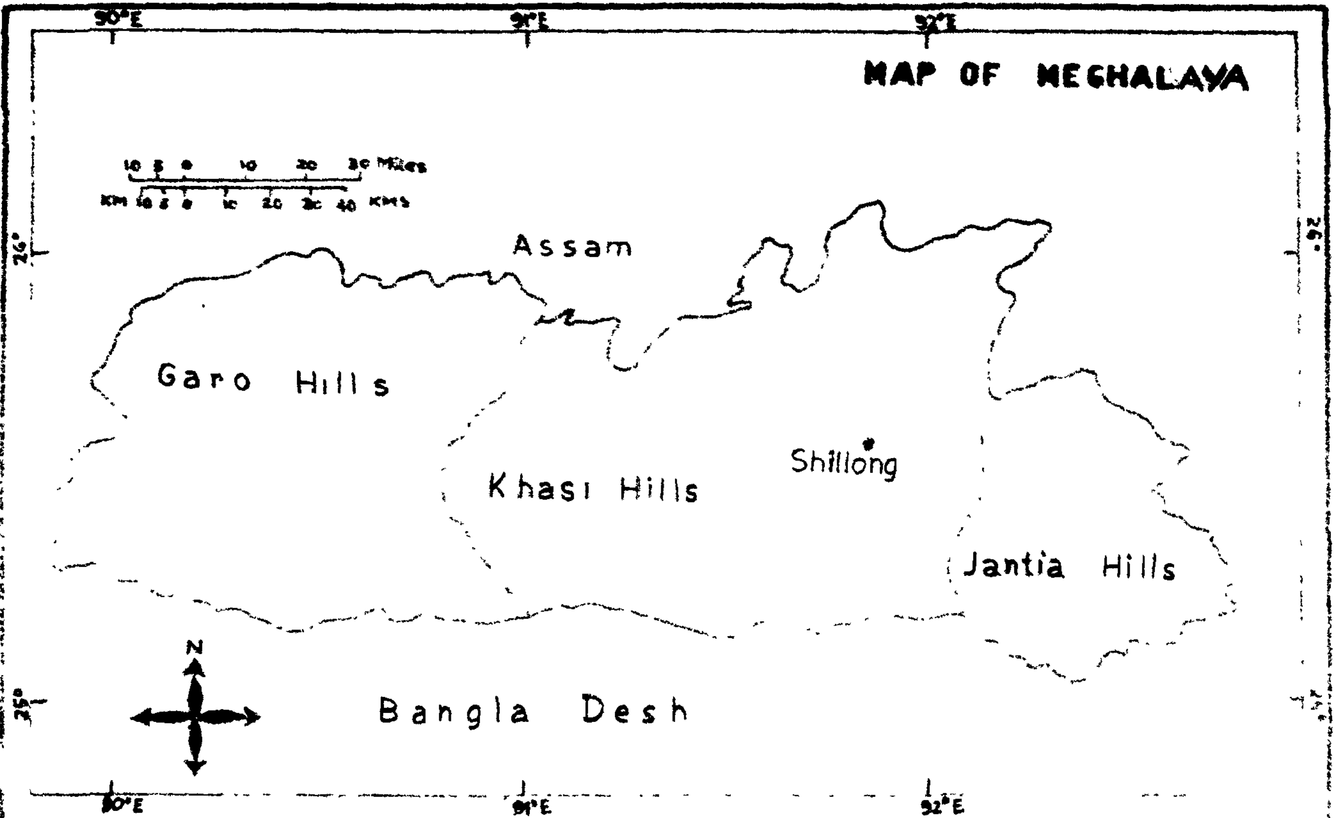
are of much importance.

The successive colonization of pine leaf litter by fungi in relation to various environmental factors is little understood. Very little information is available at present about the role of yeast, bacteria and actinomycetes in pine litter decomposition for which the present investigation was undertaken. Pinus kesiya Royle ex-Gordon was selected because of its economic importance and wide coverage in the region. The plants also produce abundant litter which are added to the soil after the leaf fall and this contributes significantly in the cycling of the nutrients. Moreover, presence of some acids that occur in pine needles (Freedi et al, 1975) may play a dominant role in litter decomposition under natural condition.

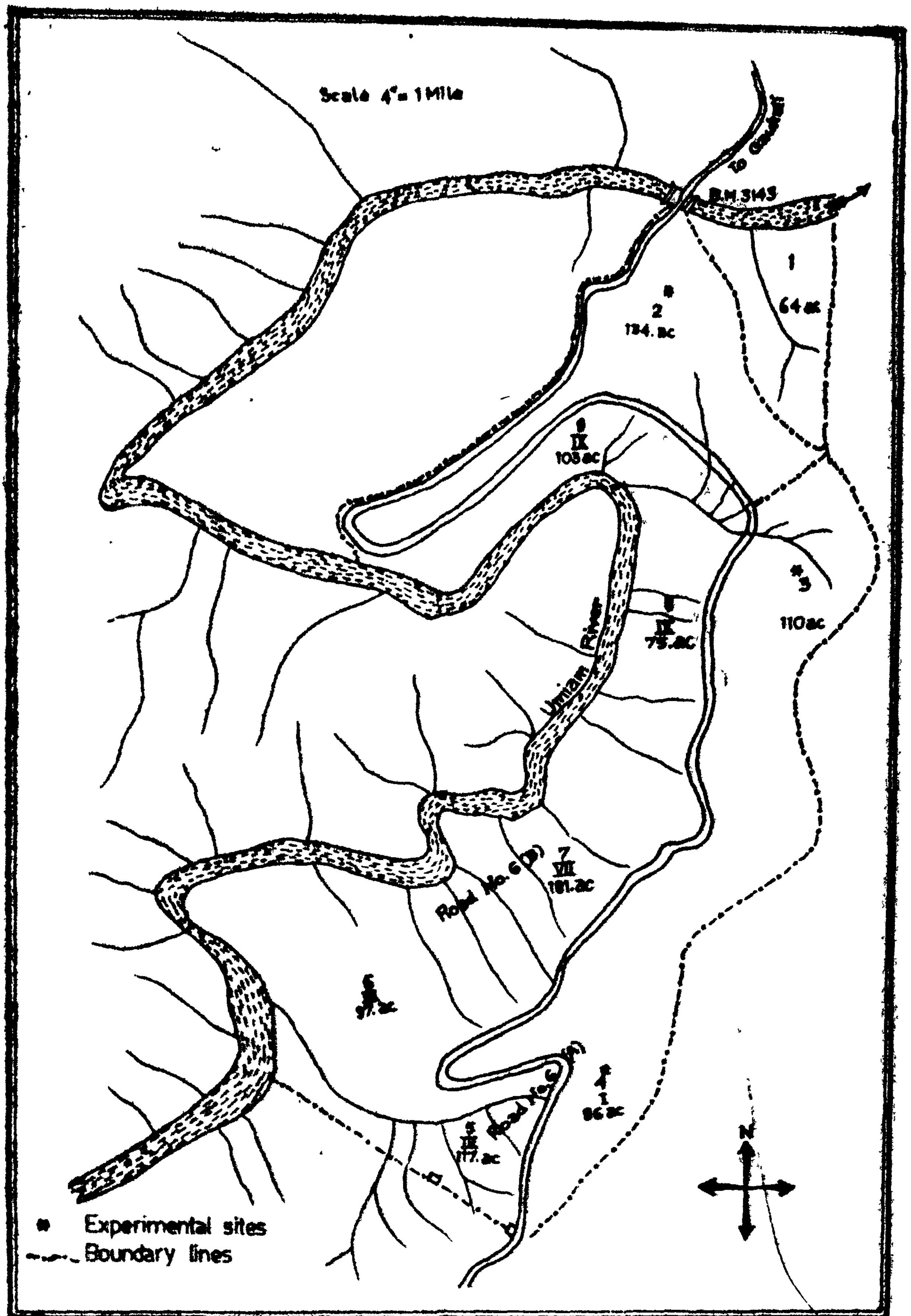
The present investigation was undertaken with a view to understand the "ecosystem function of Pine forest of Meghalaya" and the investigation was carried out in a reserve pine forest maintained by Government of Meghalaya adjacent to Shillong. The detail description of the experimental site is given below :

Description of the experimental site :-

Geographical location : The study area as shown in the map is named as "Riatkhwan reserve forest" is about 15 km from Shillong city (Latitude 25°34' N and longitude 92°47' E) on the western side of Gauhati-Shillong road. The area has an altitude range of 950 m to 1280 m MSL. Khasi pine (Pinus kesiya Royle ex-Gordon) plantations raised by the Meghalaya Government is the main vegetation of this area. The dominant pine vegetation is associated with



Map 1: LOCATION OF SHILLONG IN MEGHALAYA



Map 2: RIATKWAN RESERVE FOREST, SHILLONG (enlarged)

certain weeds. It occupies a narrow zone just above the Barapani lake. The slope of these hills is adjacent to Umiam river as shown in the map (2). The study area is divided into several compartments and the present work was carried out in the compartment numbers 2,3 and 4 as shown in the map(2). Compartment number 2 consists of the plant species sown in the year 1961 on an altitude of 950 m MSL. Compartment number 3 at an altitude of 1190 m MSL consists of pine plantation also of the same age. Compartment number 4 consists of three pine plantations of the year 1955, 1965 and 1970 at an altitude of 1250 m MSL, 1200 m MSL and 1280 m MSL respectively.

Physiographically, the area represents a remnant of an ancient plateau of Pre-cambrian India peninsular shield block uplifted to its present height. The Kermel of the plateau is the exposed Archaean gneisses and phyllites intruded later by younger granites and basic/ultrabasic suites. This ancient surface of the plateau is still preserved with marks of different cycles of denudation. It is hidden beneath the Mesozoic traps along the central Southern fringe and Cretaceous Tertiary and post Tertiary sediments. The present physiographic configuration of the plateau was attained through different geological events since Mesozoic to present day as indicated by the polycyclic erosional surface at various levels.

The Shillong plateau is a horst which has been block uplifted since zurassic times to its present height of 610 to 1544 m above M.S.L. and its tactonic history begins with the effusion of plateau basalts (Sylhet traps)

through fractures and faults in the basement and uplift and subsidence of adjacent basement blocks. These were followed by upper Cretaceous Tertiary sedimentation into the relatively down thrown portions along faults. The tactonic force has been vertically dominated and controlled by differential movements along these basements fractures.

Vegetation :- A list of the plants including undergrowths present in the area of study is detailed below. The plants were almost the same for all the plantations studied.

Trees : Pinus kesiya Royle * ex Gordon
Schima wallichii

Herbs : Eupatorium adenophorum*
Pouzolzia zeylanica
Urena lobata
Oxalis corniculata
Oxalis corymbosa
Frageria indica
Rubus ellipticus
Commelina benghalensis
Oldenlandia burmanniana
Hedychium coronarium
Erigeron linifolius
Scutelaria bicolor
Anemone rivularis*
Hypochaeris radicata

Shrubs : Lantana camara
Desmodium triflorium
Osbekia crinata

Grasses : *Setaria glauca**

*Capillipedium assimile**

*Imperata cylindrica**

*Paspalum dilatatum**

*Digitaria adscendens**

Saccharum officinalis

Eriantnus sps.

Poa annua

* Indicates dominant plants.

Environmental condition :- The region experiences a tropical monsoonic climate, the summer temperature recording as high as 28°C and the mean winter temperature falling down to 6°C with periodic fluctuations below the freezing point, marked by appearance of ground frost at night and morning. The average annual rainfall of the state is around 205 mm. The periodic climatic data is represented in the Fig.13.

CHAPTER I

SEASONAL VARIATION IN THE POPULATION AND ACTIVITY OF THE SOIL MICROORGANISMS IN THE PINE FOREST

INTRODUCTION

One of the most striking features of the soil microflora is the diversity. Actinomycetes, fungi and bacteria, belonging to innumerable genera and species, can be found in almost any soil sample. The relative proportions of the different groups are influenced to some extent by the environment.

The geography and ecology of soil microorganisms have not been well studied until recently. The paucity of research is evident in a fundamental monograph by Alexander (1971). The author could find very limited information on the subject. However, the attempt of a number of scientists to ascertain patterns of distribution of soil microorganisms ended in failure. Inspite of the exciting development of microbiology in the last 100 years, there may be little doubt that our understanding of microbial life in complex natural environments, particularly in soil, is far from being based on principles of this art of science. Microbiological studies have been carried out mainly with pure culture under well defined conditions, and many microbiologists dislike complex natural environment. However, natural environment such as soil offers great challenge to microbiologists enquiring in its dynamic features.

In contrast with pure cultures, in which attention has been focussed mainly on the chemical composition of their media, the physical conditions of the environment play prominent role in the case of soil microorganisms. Soil is a heterogeneous, discontinuous environment and is dominated by a solid phase varying in size from less than 0.2 mm to greater than 2 mm. This discontinuity and variability in

particle size may result in a composite of enumerable small microbial communities, each circumscribed by its own immediate environment, as suggested by Stotzky (1974).

Soil is essentially a colloid dispersed system. The colloid size particles play an important role in determining the physical or physico-chemical properties of microbial environment in soil. The most important characteristic of a colloidal system can be ascribed to the fact that the ratio of surface area to volume of the particle is extremely large. Soil particles are divided into various fractions as shown below :

Stones	Gravel	Course sand	Fine sand	Silt	Clay
20.0	2.0	0.2	0.02	0.002 mm.	

The relative proportions of each of these size fractions in any one soil are defined as the soil texture. Soil colloid particles which are reactive consist chiefly of clay minerals. Clay minerals are constituted of distinctly crystalline minerals which are composed of unit of aluminia and silica. Most clay minerals are platelike in character. Their electron micrographs show that Kaolinite has sharp and well defined edges and montmorillonite has structures ranging from an amorphous appearing material to extremely thin plates. The clay lattice carries a net negative charge as a result of isomorphous substitutions of certain electropositive ions by such cations of lower valence. Consequently, the flat layer (face) surface of a clay particle has a constant negative charge. Colloidal particles

of clay are usually charged negatively. To balance the negative charge, the particles tend to absorb counter ions (cations), which are governed by many factors. The most important factors are type of cations and the clay particle. According to Stotzky _et al_ (1966) respiration of various bacteria can be stimulated by montmorillonite and also although to a lesser extent by Kaolinite, which may primarily be the result of clays maintaining the pH of the environment at levels suitable for microbial life. Stotzky (1974) emphasised that a single environmental factor, such as the type of clay minerals, will greatly influence microbial life in soil. Outstanding examples of such influence are the correlation between the rapid spread of _Fusarium oxysporum_, _F. cubence_ and _Histoplasma capsulatum_ in certain soils. Clay particles also play important role in the survival of bacteria in soil. Dommergues (1964) showed a protective effect of a clay on survival of _Azotobacter vinelandii_, _A. chroococcum_ and _Beijerinckia indica_ at low humidity was markedly enhanced by addition of Kaolinite. Stotzky _et al_ (1966) showed that microbial activity is depressed at a higher salt concentration, but the depression is lessened by addition of montmorillonite.

One of the essential characteristics of soil microhabitat is its discreteness, the chemical, physical and biological characteristics of the microhabitat differ widely from point to point or from time to time. The physical constitution of a soil material is determined by the size, shape and arrangement of the solid particles. The size and distribution of pores in soil are among the most important

factors determining microbial life, since micro-organisms live exclusively in pore space, and retention of water indispensable to microbial life is closely related to the size of each pore.

The concept of capillary pore as the most favourable microhabitat of soil bacteria is also supported by several observers. Seifert (1965) observed that nitrification was stimulated by increase of water content until PF 2.7 and further increase of water content did not result in stimulation of the activity. Tanaka et al (1972) showed that the number of bacteria increased proportionally to water content of soil until it attained a critical value which was specific to each soil. With a larger amount of water than the value, the number was not affected distingtly by water content. When large noncapillary pores are saturated with water, oxygen diffusion into soil aggregate may become very difficult; consequently bacterial activity and growth may be markedly depressed. Soluble substances in large pores may be eluted by rain or irrigation; thus nutrients indispensable for bacterial growth may be apt to be wanting. Noncapillary pores probably larger than 6 mm, may not be as favourable microhabitat as capillary pores, even if water is sufficiently supplied.

Several studies have been carried out in different parts of the world to correlate biological activities of soil to their fertility status. Among them the important ones are : total microbialcounts, rates of nitrification, ammonification, cellulose decomposition and individual enzyme activities, such

as urease, invertase and amylase. All these parameters have their own drawbacks and none of them alone predicts the overall biological activity. The plate counts give the estimation of microbial population which is again dependent on the type of medium used. The biochemical and enzymic methods measure only certain metabolic capacities of selected groups of microorganisms. These drawbacks led the workers to turn to the methods like carbon-dioxide evolution, oxygen uptake and dehydrogenase activity. Dehydrogenase activity measures the net effect of numerous enzymic processes taking place in the soil. It is regarded as being more dependent on the general metabolic status of the soil microflora than the activity by specific cell free enzymes acting upon certain substrates. Determination of dehydrogenase activities has, therefore, been used since 1956 when Lenhard introduced the method with triphenyltetrazolium chloride (TTC) as an artificial hydrogen and electron acceptor.

Rate of carbon dioxide evolution from forest soil reflects the metabolic activity of the biota, including live roots and organisms responsible for decomposition. One of the parameters indirectly affecting the forest productivity is the decomposition of organic matter on the forest floor and in soil. The enzymatic breakdown of organic matter by litter and soil organisms, mostly bacteria and fungi, ultimately releases the nutrient elements which may then be taken up by plant roots and are recycled. Carbon dioxide (Co_2), which is concomitently released with nutrient elements during decomposition, may be monitored as an indication of mineralization rates (Witkamp, 1971). As the climatic fluctuations in this north eastern part of the country are

very significant, it was felt necessary to investigate these two methods simultaneously to compare which methods would be more appropriate to assess the biological activity of the acidic soil of this region.

Soil microbiology has engaged the attention of several researchers, but the ecological factors governing the distribution of soil microbe have been studied by a few modern workers. Distribution of soil microbes in different seasons of the year and the effect of the various factors, climatological and edaphic governing their distribution have not received adequate attention by the soil microbiologists of this country.

With the existing knowledge it was not possible to understand the nature and activities of the soil microbes of this region in different seasons.

The ecology of soil microorganisms is much more difficult in the sense that the methods of study are much more labour-consuming than the ecology of higher plants. Hence the present work which is first of its kind in this region, was undertaken with a view to understand the nature and activities of the soil microbes of the pine forest soil and their role in litter decomposition.

REVIEW OF LITERATURE

Soil microflora - Waksman (1952) indicated, "among the various factors which influence the abundance of microorganisms in the soil, the most important are organic matter, soil reaction, moisture, temperature, aeration and nature of crop grown. The distribution of microorganisms in the soil is, therefore, controlled by numerous ecological factors, comprising climatic or atmospheric, edaphic or soil, and biotic or living.

Successful attempts to describe the ecology of soil fungi in relation to higher plants have been made by the studies of Tresner et al (1954) on a forest continum and by Orpurt and Curtis (1957) on Praire continum of Wisconsin.

Saksena (1955) and Warcup (1957) found a marked decrease in viable propagules of soil in summer, subjected to extreme desication at that time of the year. The former, further, observed that moisture, as a rule, was favourable for the growth of fungi as long as there was no water logging. Kluyver et al (1956) studied the distribution of microorganisms in soil and described that the cosmopolitan distribution of microorganisms does not mean that they occur everywhere in large numbers. Their active reproduction is favoured by certain conditions only. It seems that in different soil types, varying groups of microorganisms predominate. England and Rice (1957), while making a comparative study of soil fungi of a tall grass Prairie with that of an abandoned field in central Oklahoma, observed that although a few species appeared with high frequency throughout the year, most species were of seasonal or sporadic nature. It means that species composition of each

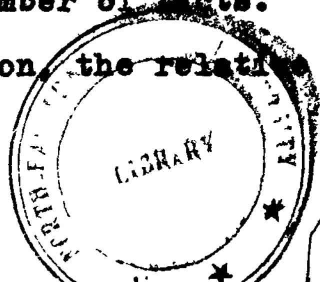
plot changes from season to season and to a lesser degree from month to month.

Ordin (1958) studied the influence of vegetation on the microflora of soils and concluded that different types of soil harbour different dominant fungal species. Ivarson and Katznelson (1960), while working on the rhizosphere microflora of yellow birch seedlings, noted that there was a pronounced rhizosphere effect on the bacterial population while other groups like actinomycetes and fungi were less affected.

The soil fungi of Conifer hardwood forests were studied by Christensen (1960) in Wisconsin and he concluded that species composition of the soil microfungal communities was correlated with the species composition of the cover vegetation.

Parkinson and Kendrick (1960) investigated soil microhabitat in relation to soil fungi and opined that the conditions prevailing in very small spaces in soil will have a decisive influence on growth and reproduction of soil fungi.

The effect of geography on the distribution of microscopic fungi in soil has been considered in a number of studies. Some points relating to the distribution of microscopic fungi in soil have been generally recognized. During the studies of soils of different zones of USSR a considerable amount of information on fungal ecology was collected by Mishustin _et al_, (1960). An analysis of their results allows for the establishment of a number of facts. Thus in soils with nonintensive mineralization, the relative


LIBRARY

abundance of fungi of the genera Penicillium and Mucor increases. Further, to the south zones, fungi of the genus Penicillium are replaced by the fungi of the genus Aspergillus. It should be noted that the relative abundance of Aspergillus not infrequently increases in alkali soil located in the chermozem zone. Mucor strains that prefer organic nitrogen are common in the upper layers of virgin soil containing many nondecomposed plant residues. Mucor ramanianus is found only in forest soils. Representatives of the genus Choanephora occur only in Sierozems. In soils with less intensive biochemical changes, the biochemically less active representatives of the genus Penicillium predominate. In soils with less favourable conditions, say, in alkaline, saline and desert soils, fungi of the monoverticullata section dominate. The section biverticullata is chiefly largely represented in forest soils. The section asymetricullata is widely distributed in most parts of cultivated soils. Representatives of the genus Fusarium are concentrated in soils covered by grass vegetation. Forest soils are practically free from these fungi. Thus it can be concluded that the composition of different fungi undergoes different regrouping in different soil types.

Wright and Bollen (1961) while working on microflora of Douglass fir forest soil, observed that there was extensive monthly fluctuation in number of microorganisms and the latter greatly increased during the rainy periods and decreased during drought.

Tyeplyakova (1961), studied the vertical zonation of the soil microflora of Ala Taumountains of Kazakhstan, the

number of pigmented actinomycetes defreases as the altitude of soils above the sea level increases. Thus <u>Actinomyces fumoses</u>, <u>A.actinoides</u> often occur in mountain meadow soils, while they are largely absent from Sierozems. Some species of actinomycetes, such as <u>A</u>. <u>globisporus</u> and <u>A</u>. <u>griseus</u> occur in all types of soil.

Christensen <u>et al</u> (1962) studied the soil microfungi of West mesic forest in Southern Wisconsin and observed that there existed qualitative and quantitative difference in species composition among the stand, particularly between the two stands representing extremes in vegetational composition.

Williams (1963) studied the distribution of fungi in the horizons of a podzolized soil and concluded that even under an apparently uniform plant cover considerable variations in the microbial population are observed.

Griffin (1963) reviewed the effect of soil moisture on soil fungal population and concluded that nearly all fungi will be able to exert the necessary force to absorb water and to grow without impediment even in drier soils. However, in soils drier than permanent wilting point, this ability will be different for different fungal species. Further, moisture content, texture and structure, which affect soil aeration, have significant influence on fungal activity. Soil microfungi of open bogs and Conifer swamps in Wisconsin were studied by Christensen and Whittingham (1965). Soil fungi from three areas representing successional stages in bottomland forest vegetation in Central Oklahama were compared throughout the year at two months interval by Mallik and Rice (1966). The numbers of accompanying bacteria and

actinomycetes were also determined. They found a good correlation between soil fungi and three successional forest communities. The number of fungal species was low in the pioneer area and slightly higher in the transitional area than in the climax. Apparently the maximum number of fungal species attained during succession is reached before the climax stage.

Saito (1966) studied the soil actinomycetes and observed that actinomycetes may increase briefly when fresh organic matter is added to the soil, as at leaf fall or when crop residues or farmyard manure are ploughed in. However, they often colonize new substrates slower than bacteria and fungi.

Seasonal fluctuation in number of soil microorganisms in different climatic zones of the USSR was reported by Mishustin (1966). For obvious reasons, the number of non-spore forming bacteria showed the greatest variation during the course of a season. The population fluctuations among the bacilli were less marked. Different geographic and edaphic factors appreciably affected the number of actinomycetes and microscopic fungi. It is clear from this report that soils of Southern Zones of USSR are the richest in microorganisms. The somewhat high count in the virgin soils of the extreme north can be linked to the accumulation on the surface of these soils of partially decomposed plant residues, which in the warm periods of the year form a godd substrate for microorganisms. In a still more southern zone, the number of fungi decreases. Due to increasing competition of bacteria and actinomycetes with the fungi, the number of the latter

decreases in the soils, specially those characterized by neutral reactions.

Mishra (1966) studied the seasonal variation in fungal flora of grasslands of Varanasi (India) and recorded a seasonal effect on the prevalence of different fungal species. He further observed (1967) a good correlation between soil fungal population and temperature and moisture content.

Soil is a poor medium for microbial growth and the high organic matter has a large effect on the number of microorganisms. Rangaswami et al (1967) established a correlation between organic matter content and number of actinomycetes in soils from South India.

Soil types of some mountain ranges in the Caucasus and Middle Asia were studied by Mishustin and Mirzoyeva (1968). They found that the effect of altitudinal zonality on the composition of soil microflora is the same as that of latitudinal zonality. Higher in the mountains, where the average annual soil temperature is lower, both the number of bacteria and the percentage of Bacilli and actinomycetes decreases. Some soil types have different profiles and distribution of organic matter. These differences affect the microbiological profile of different soils.

During the investigations of the effect of various preceding crops on the soil fungus flora in wheat fields, Domsch et al (1968) obtained sufficient data to draw general conclusions about the distribution of certain species in the soil. Domsch and Gams (1969) studied the variability and potentiality of a soil fungus population to decompose pectin

Xylan and Carboxymethyl cellulose to determine the ability of certain soil fungi to degrade different substrate.

Christensen (1969) studied the soil microfungi of dry to mesic Conifer hardwood forest in Northern Wisconsin. His findings confirmed the hypothesis that species composition in soil microfungal communities is correlated with vegetation. Gray (1969) studied the numerical taxonomy of soil bacteria in pine forest soil and evaluated the importance of such studies for identification.

Apart from the effect of climate and vegetation on soil microflora it has been shown by Mai (1970) that the transformation of organic and mineral substances is always accompanied by microbial succession. Hence it can be concluded that organic matter transformation in the soil must promote the development of different microbial associations. Apart from the geographic factors, each zone is certainly defined by its environmental conditions, Davies and Williams (1970) attempted to correlate the distribution of actinomycetes population as a whole and also of individual isolates to various environmental factors in a developing podzol. They studied the quantitative and qualitative nature of actinomycetes population in the mineral horizons. From their results, depth, pH and moisture levels of the soil appeared to exert varying influence on different isolates. Horizons could not be sharply defined by the actinomycetes flora. During last century since actinomycetes were discovefed they have shown to be an important producer of antibiotics, yet their study has barely advanced beyond the stage of enumeration and identification. Much remains to be discovered of their ecology, interrelatbons

with other organisms and importance in the degradation of soil organic matter. There are few comparisons of the actinomycetes populations of different soil types.

In India no work has been done on the ecology of soil actinomycetes in acidic soil like that of pine forest soil. Actinomycetes usually prefer neutral or slightly alkaline soil; few are able to grow in soils more acidic than pH 5. Williams _et al_ (1971) reported a group of _Streptomyces_ sp isolated from acid soils that could grow in culture at pH 3.5 - 5.5, spore chains with similar morphology grew on pine needle fragments in the acid litter layer.

Srivastava and Mishra (1971) in their studies into rhizosphere microflora of certain medicinal plants, observed that the total free amino acids in root has a direct correlation with the quantity of rhizosphere microflora. Mishra and Kanaujia (1973 a) investigated rhizosphere mycoflora of certain gymnosperms in relation to different seasons and observed that fungal population in different gymnosperm plants was different. They also observed a good correlation between amino acid contents of the root of _Aurocaria_ species and number of fungi present on the root. They (1973 b) further studied the distribution of soil fungi in relation to cover vegetation and physicochemical characters of the soil collected from different places of North India and observed that fungal population was generally higher in cultivated fields and nature and duration of cropping affected the population. It was also suggested that moisture content, organic matter content, and pH of the soil affected fungal flora.

Wicklow and Whittingham (1974) investigated fungal population inhabiting the organic and mineralised soil horizons, and evaluated the effect of catastrophic vegetational disturbances on their species composition in selected upland forests in Northern Wisconsin. Baker (1974) investigated the microbiology of the soils of Subalpine heath, a palsa peak and two iron podzols in Utsjoki.

In India Joysheela and Oblisami (1975) reported the prevalence of rhizosphere microflora of certain medicinal plants and emphasized on plant microbe interrelationships. Gray (1976) studied the survival of vegetative structures of microbes in soil and stressed on the nature of environment on the survival of the microbes.

Dehydrogenase activities and Carbon dioxide evolution -

Level of dehydrogenase activity in soils is considered to give some guide to the microbiological activity of the soils.This aspect has been investigated intensively since the introduction by Lenhard (1956) of a method for the estimation of this activity. Dehydrogenase activity appears to be more dependent upon the metabolic state of the the microbial population of the soil than upon the activity of specific free enzymes on particular substrates.

Stevenson(1959) and Casida(1964) used the 2,3,5 triphenyl tetrazolium chloride(TTC) reduction method in an attempt to equate the dehydrogenase activity with the biological activities of the soils.

Skujins and Mclaren (1968) studied the dehydrogenase activity in some air dried soils that had been stored for a

few years. Ross (1970) studied some factors influencing the estimation of dehydrogenase activities of pasture soils in New Zealand. Ross et al (1970) further investigated the enzyme activities and oxygen uptake of soil under pasture in temperature and rainfall sequences. They further (1973) studied the invertase and amylase activities in a soil profile and found that both the activities were mostly positively correlated with oxygen uptake , particularly in the presence of added glucose.

Baker (1974) studied the soil microbial activity in Finish Lapland by measuring the levels of dehydrogenase and B - fructo furanosidase by laboratory respirometry and the acetylene reduction technique for nitrogen fixation. Hast (1974) from Swedish Coniferous forest reported the total activity of microorganisms in protein amended soil. He determined this aspect by plate counts, respiration measurements, dehydrogenase activities and ATP analysis. ATP analysis was a better measure of the activity of the microbial population rather than the biomass study.

In India very little information is available on this aspect of soil microbiology. Vishwanath et al (1975) studied some selected soils to determine the contribution of bacteria, surviving chloroform and toluene treatment with regard to dehydrogenase activity.

Wingfield (1977) studied the effect of the application of herbicide Dalapon on bacterial population and dehydrogenase activity in soil of Begbroke of Hill Yarton.

Witkamp (1966) studied the evolution of Co_2 from forest floors of pine and maple stands.

Variables which have been shown to effect the Co_2 evolution from soil include temperature and moisture (Katznelson and Stephenson, 1956; Witkamp, 1966; Reiners, 1968; Garett and Cox, 1973), pH (Katznelson and Stephenson, 1965) and substrate (Katznelson and Stephenson,1956; Witkamp, 1966). Determination of rates of Co_2 evolution from forest floors is, therefore, essential to the construction of ecosystem carbon budgets (Wood Well and Botkin, 1970; Reichle _et al_, 1973).

Garrett and Cox (1973) reported that moisture had the greatest limiting effect on Co_2 evolution from the floor of an Oak hicory forest in Missouri and that temperature was most limiting in the spring and winter.

Edward (1975) discussed the use of moisture and temperature to predict Co_2 evolution rates from seperate litter and mineral soil horizons in a mixed deciduous forest in Eastern Tennessee. Mortansen and Staff (1975) in Swedish coniferous described two methods of evolution of heat and Co_2 for measuring the total activity of organisms in soil. In India, not much work has been done on this aspect of soil microbiology to understand the complex forest ecosystem.

MATERIALS AND METHODS

A. <u>Selection of the site</u> :-

The investigation of the soil microflora was carried out for two years, starting from April 1976 - April 1978. During 1976-1977, the investigation of the soil microflora was carried out under five selected pine plantations viz. 1955, 1961 (plantation on high altitude), 1961 (plantation on low altitude), 1965 and 1970. The details of the plantations are given in the chapter general introduction. The five plantations were selected to investigate the variation in the microbial population amongst plantations of different age. After one year of study of the soil microflora of these plantations, when no significant variation was observed between them further investigation of the soil microflora during 1977-78 was extended for only two pine plantations viz. 1955 and 1970.

B. <u>Soil sampling procedure</u> :-

The first sampling of the soil from different plantations mentioned above, started on 5th.April,1976 and the subsequent samplings were done at one month interval for a period of one year.

Soil samples were collected by inserting a sterile bowel vertically into the soil to a depth of 15 cms. at five randomly selected sites within each stand. Each set of five samples was thoroughly mixed in a sterile polythene bag in an effort to minimize local variation in the microbial population, and the samples were transported to the laboratory immediately. All aseptic precautions were taken to avoid contaminations. Samples were kept in the laboratory freeze at a temperature of +3°C until the plate counts were

done. Additional bulk of samples collected from each site and from each plantation provided material for pH and moisture determinations. The same procedure of the soil samplings was followed for the investigation of soil microflora during May 1977 - April 1978. Atmospheric temperature, rainfall and humidity were recorded throughout the sampling periods.

C. Isolation of Soil Fungi :-

Warcup's soil plate method (Warcup, 1950) was followed for isolation of fungi from the soil samples. Small amount (0.003 g.) of soil was taken from the main samples by mean of a sterile nichrome spatula having a flattened tip. The soil was then dispersed in a few drops of sterile water in the bottom of the sterile petridishes. Eight to ten ml of melted and cooled Martin's rose bengal agar medium supplemented with streptomycin sulphate was poured and the soil particles were dispersed throughout the medium by shaking and rotating the dishes. Three replicates were maintained for each sample. Same method of isolation was followed for all the plantations. The plates were then incubated upside down at a temperature of 25 ± 1°c for 5-6 days in BOD incubator and were examined carefully everyday to remove the fast growing forms. The number of fungal colonies was counted with the help of a digital colony counter and the fungi/g dry soil were calulated by taking into consideration of the moisture content. Isolation and purification was carried out in a laminar flow chamber.

The isolation and purification of fungi for detailed study were done either by single spore method or by cutting

of hyphal tips. The pure cultures were transferred to fresh agar slants of Malt extract and Czapek's Dox agar medium and preserved in the refrigerator running at 10°C. For identification of the most of the fungi the books consulted were those of Gilman (1956). Barnett and Hunter (1972), Subramanian (1971).

D. Isolation of bacteria and actinomycetes :-

Dilution plate method was followed for the isolation of bacteria and actinomycetes throughout the sampling period. 10g of soil from each sample was taken and put in 250ml sterilized conical flask and a soil solution in sterilized distilled water of 1:10 dilution was prepared. Further dilutions viz., 1:100, 1:1000 and 1:10,000 were prepared with sterilized distilled water for each sample. The stock solutions were always thoroughly hand shaken for 10-15 minutes before making any dilution. 1:10000 dilution was finally considered suitable for isolation of bacteria and actinomycetes. Throughout the investigation period the same dilution (1:10,000) was used for the various samples. Isolation of bacteria was done on nutrient agar medium containing beef extract whereas actinomycetes were cultured on starch casein agar plus antibiotics medium which has been shown to be highly selective for these microorganisms (Kuster and Williams, 1964). The selectivity of this medium for actinomycetes was further increased by the addition of antifungal antibiotics, actidione and nystatin each at a conc of 50 mg/ml of medium (Williams and Davies, 1965). 0.5 ml of soil suspension from 1:10,000 dilution was transferred aseptically to previously

LIBRARY

poured nutrient agar medium and starch casein medium for the cultivation of bacteria and actinomycetes respectively. Seperate pipettes were used for transferring the soil solution in each case to avoid contamination. Isolation was carried out in sterilized lamminer flow chamber throughout the investigation period. Three replicate plates were maintained in each case. The petriplates were shaken gently to disperse the inoculum uniformly over the surface of the agar medium. The plates were incubated upside down at a temperature of 30 ± 1°C in a bacteriological incubator. The plates for bacterial counts were incubated for 24 hours whereas the plates for actinomycetes were incubated for 7 days only. Number of bacteria and actinomycetes colonies was counted with the help of a digital colony counter and their population per g dry soil was calculated by taking into consideration of the moisture content. In this study only the bacterial and actinomycetes population was considered and no attempt was made to identify the species. Pure cultures of bacteria and actinomycetes were transferred to fresh agar slants and preserved in the refrigerator running at 10°C. Same methods of isolation of soil microflora was followed for the investigation of soil microflora during 1977-78.

E. Composition of the media used for the isolation of fungi, bacteria and actinomycetes :-

a) Czapek's Dox agar - (Raper and Thom, 1949).

Agar	-	15.0 g.
$Na\ No_3$	-	2.0 g.
$K_2H\ PO_4$	-	1.0 g.

$Mg\ So_4\ \ 7H_2o$	-	0.5 g.
Kcl	-	0.5 g.
$FeSo_4\ 7H_2o$	-	10.0 mg.
Sucrose	-	30.0 g.
Water (distilled)	-	1000 ml.

b) Malt extract agar

Agar	-	25,0 g.
Malt extract (Difco)	-	20.0 g.
Dextrose	-	20.0 gm
Peptone	-	1.0 g.
Water (distilled)	-	1000 ml.

c) Peptone-dextrose-Rose bengal agar (Martin 1950).

Agar	-	20.0 g.
KH_2Po_4	-	1.0 g.
$MgSo_4\ \ 7H_2o$	-	0.5 g.
Peptone	-	5.0 g.
Dextrose	-	10.0 g.
Rose bengal (1%)	-	3.3 ml.
Distilled water	-	1000.0 ml.
Streptomycin	-	30.0 mg.

<u>Medium for Bacteria</u> -

<u>Nutrient agar</u> -
(Difco manuel, 1953).

Agar	-	15.0g.
Beef extract	-	3.0 g.
Peptone	-	5.0 g.
Nacl	-	8.0 g.
Water (distilled)	-	1000.0 ml.

Medium for Actinomycetes -

Starch-Casein agar (Kuster and Williams, 1964).

Agar	-	18.0 g.
Starch	-	10.0 g.
Casein (Vitamin free)	-	0.30 g.
KNo_3	-	2.0 g.
Nacl	-	2.0 g.
K_2HPo_4	-	2.0 g.
Mg So_4 $7H_2o$	-	0.50 g.
$CaCo_3$	-	0.02 g.
$FeSo_4$ $7H_2o$	-	0.01 g.
Water (distilled)	-	1000.0 ml.
Nystatin and Actidione	-	50 Mg/Ml each.

F. Determination of percentage relative abundance and percentage frequency of fungi :-

The percentage relative abundance of a fungal species was calculated from the formula as detailed below:

$$\frac{\text{Total number of individual species of fungus}}{\text{Total number of individuals of all species}} \times 100$$

The term 'frequency' is used to refer the frequency of a fungus during the entire sampling period and is represented by the number of samplings in which the fungus appeared, expressed in percentage. Based on percentage frequency the fungi are grouped as 'dominant' 81-100%; Common 61-80%;frequent 41-60%; occasional 21-40%; and rare 1-20% (Vittal, 1975).

G. Seasonal record of the fungal population :-

Seasonal record of the fungal population,was made in

three seasons viz., (a) summer, from April to June (b) rainy, from July to October and (c) winter, from November to February. The fungi recorded during the different months of the seasons were consolidated and percentage frequency was calculated by averaging the monthly data described earlier.

H. Determination of dehydrogenase activity, CO_2 evolution and microbial population of the forest soil :-

The experiment was conducted for a period of 1 year starting from May 1977 to April 1978 under a selected pine plantation viz: 1970 plantation. Soil from this selected pine stand was collected regularly at one month interval for a period of one year. During each sampling top soil upto depth of 15 cm from five randomly selected spots were collected as described earlier. Each set of five samples was thoroughly mixed in a sterile polythene bag in an effort to minimize local variations in the microbial population and the samples were transported to the laboratory immediately. All aseptic precautions were followed to avoid contamination. The samples were used on the same day to determine dehydrogenase activity. Additional bulk of samples collected in each case provided material for pH and moisture determinations. Soil temperature was always recorded during the entire sampling period. Organic matter, organic carbon and C/N ratio also were determined. Atmospheric temperature, rainfall and humidity were recorded throughout the investigation period.

Dehydrogenase activity of the soil was determined by 2,3,5 Triphenyltetrazolium Chloride (TTC) reduction technique as modified by Casida et al (1964). To determine dehydrogenase

activity (DHA) 5.0 g of soil sample (free from any plant debris) was dispensed in six test tubes. To each tube 0.1 g of $CaCO_3$ and 1 ml of 1.5% aquous solution of TTC were added in succession. Three of these tubes received 1 ml of 1% solution of glucose and 3 ml of distilled water. The quantity of liquid was found to be enough to saturate the soil and to form a liquid layer on the soil sample. This ensured adequate anaerobiosis for TTC reduction. The content of each tube was well mixed, plugged with a rubber stopper and incubated at 37°C for 24 hours. The TTC was reduced to Triphenyl formazan which was extracted with methanol. The soil from each tube was quantitatively transferred to Whatman No.40 filter paper with the help of methanol and washed down into a 50 ml. volumetric flask, with small portions of methanol till the filtrate ran free of pink colour. The volume of the filtrate was made up to the mark with methanol. The OD (Optical Density) due to pink colour of the filtrate was determined at 485 mμ by means of a spectrophotometer (Spectronic-20) using methanol extract from the control (without soil) as the blank. The results are depicted on the basis of O.D. taken at 485 mμ.

Inverted box method was used to determine CO_2 evolution from the forest floor (soil) (Walter and Haber, 1952). Glass beakers of 100 ml. capacity containing 50 ml of 0.1 N KOH solution was covered with tin containers (5071.2 cms. sq.area) and exposed to forest floor for 24 hours under natural conditions. Control sets were used as follows : a small area of the forest floor (according

to the size of the tin used) was covered by means of a polythene sheet to ensure no Co_2 accumulation took place from the forest floor and the whole system along with 0.1 N KOH solution was covered by inverted tin. After 24 hours of exposure the amount of Co_2 fixed by the KOH solution was measured by titrimetric method using 0.1 N HCL and Phenolphthelin as an indicator. Three replicates were used in each case. Finally Co_2 evolution was expressed in $mg/m^2/hr$.

The microbial population of the soil samples was also assessed as described earlier during the determination of dehydrogenase activity and Co_2 evolution and during each sampling period.

I. <u>Analysis of the physicochemical properties of the soil</u> -

The following physicochemical properties of the soil were determined during different periods of samplings.

a) <u>Moisture content determination of soil samples</u> :-

Moisture content of each soil sample was assessed by oven dry weight method. During each collection ten g of each sample of freshly collected soil was dried at 105°C in a hot air oven to constant dry weight and from the resulting weight losses the percentage moisture content was assessed.

b) <u>pH determination of soil samples</u> :- pH of the soil samples was also determined for a period of two years. For each sample 20 g of soil was saturated with 100 ml of distilled water and allowed for constant stirring with the help of a magnetic stirer for 20 minutes. The suspension was then filtered through Whatman filter paper No.1. The

pH of the filtrate was then taken by electric pH meter.

(c) <u>Determination of the organic carbon, organic matter, and nitrogen of the soil</u> :-

Organic matter, organic carbon and nitrogen of the soil samples collected from 1970 plantation during 1977-78 were analysed as described below :

<u>Determination of organic carbon of soil</u> :-

Walkley and Blacks (1934) rapid titration method was followed for the determination of organic carbon of the soil sampbes collected at different sampling period . The soil was digested with chromic and sulphuric acid making use of the heat of dilution of the sulphuric acid. The excess of chromic acid, not used by the organic matter of the soil was then determined by titration with standard ferrous sulphate. For each sample 0.5 g of soil was taken after passing through fine mesh(no.70)and placed in a 250 ml conical flask. 10 ml of 1 N $K_2Cr_2O_7$ solution was then pipetted in the soil, and the two were mixed by swirling the flask. Then 20 ml of conc H_2So_4 was added and mixed by gentle rotation to ensure complete contact of the reagent with the soil. The mixture was allowed to stand 30 minutes. A standardization blank was run (without soil) in the same way. The solution was then diluted to 200 ml with distilled water and 10 ml of 85% phosphoric acid, 3 drops of diphenyleamine indicator was added and the solution was titrated with 1 N ferrous sulphate. Near the end point the colour became deep violet blue and at this stage Fe So_4 was added slowly with constant shaking. At the end point the colour of the solution changed to green. The percentage organic carbon present in the soil was then

calculated as follows :

Since 1 ml of 1 N potassium dichromate corresponds to 3 mg. of carbon.

$$\therefore \% C \text{ (organic)} = \frac{B - S \times 0.003 \times 100}{W}$$

B = ml $FeSo_4$ in blank titration

S = ml $FeSo_4$ in sample

W = weight of the soil sample

The organic matter present in the soil was calculated out from the percentage organic carbon as follows :

Organic matter = % organic carbon x 1.724 where 1.724 is a constant factor (Jackson,1958).

<u>Estimation of nitrogen of soil</u> :-

Kjeldahl method was followed for the estimation of nitrogen of the soil samples (Jackson,1958). For each sample 10.0 g of soil was taken after passing through (100 mesh per in) sieve in to a 300 ml. Kjeldahl digestion flask. 20 g of Na_2So_4 plus catalyst digestion mix were then added. 35 ml of Conc H_2So_4 was also added and the digestion was followed on the Kjeldahl digestion rack with low flame for the first 10 - 30 minutes, until frothing stopped and then gradually more strongly until the sample was completely charred. The flask was rotated at intervals and heating was continued until the organic matter was destroyed and the solution was cleared. At the end of the digestion when the colour of the solution turned yellow green, the heating was stopped and the flask was allowed to cool, 200 ml of distilled water was added and the solution was cautiously mixed. The solution was then ready for the determination of

ammonium content by distillation.

The solution was then poured into the Kjeldahl distillation unit held at 45° angle with condenser and was allowed for distillation. 25 ml of 4% boric acid was taken into a 250 ml conical flask and 4 drops of bromo-cresol and methyl red indicator were added. The flask was placed at one end of the condenser fitted with a receiver tube. The tube was placed in the flask so that its end was below the surface of the boric acid in the flask. After the solution was poured into the distillation flask 40% NaoH was poured till the solution turned brown in colour and was allowed for distillation till the conical flask received 125 ml of distillate.

The boric acid was then back titrated with N/14 Hcl. At the end point the blue colour of the solution disappeared and the solution turned pink in colour.

The percentage of Nitrogen was then calculated as detailed below :

$$\% \text{ Nitrogen} = (T - B) \times N \times \frac{1.4}{S}$$

when T = Sample titration, ml standard acid

B = blank titration, ml standard acid

N = Normality of standard acid

S = Sample weight,

where 1.4 is a constant factor.

RESULTS

The experimental findings are represented in the following order :

1. Quantitative assessment of the population of fungi, bacteria and actinomycetes from soil under different pine plantations and at different sampling periods during 1976-1977 and 1977-1978 are represented in the Figs.(1-5) and the statistical analyses for the same are depicted in the Tables (1-6).
2. Records of the percentage relative abundance and percentage frequency of fungal species isolated at different sampling periods during 1976-1977 and 1977-1978 under different pine plantations are given in the Tables (7-11, 17-18) and Figs. 6-12. Statistical analyses for the same are depicted in the Tables (12-16, 19-20).
3. Distribution of the different fungal species in different seasons (E.g. summer, rainy and winter) during 1976-1977 and 1977-1978 under different plantations, on the basis of percentage frequency are tabulated in the Tables 21-24.
4. Moisture content and pH of the soil during different sampling periods under different pine plantations during 1976-1977 and 1977-1978 are given in the Tables (25,27,29,31) and the statistical analyses for the same are depicted in the Tables (26,28,30,32).
5. Meteriological data during the course of studies are depicted in the Fig.13.
6. Relationship between Co_2 evolution, dehydrogenase activities, total number of microbial population and other physico-chemical properties of soil of 1970 pine plantation

during 1977-1978 are depicted in the Table (33) and Figs. (14-15).

1. <u>Quantitative assessment of the population of fungi, bacteria and actinomycetes from the soil of different pine plantations during 1976-1977</u> :-

The fungal population of the soil under different pine plantations during 1976-1977 exhibited almost a similar picture (Fig.1). It was observed that the fungal population in the soil of 1955 and 1965 plantations increased in the beginning (i.e. in the month of April-May) and a sudden fall in the population was recorded thereafter (Fig.1). In both 1955 and 1965 plantations the soil fungal population exhibited an increasing trend from the month of July onwards upto October and decreased thereafter till January (Fig.1). An increase, however, was again recorded from February to March. In both the plantations least fungal population in the soil was recorded in the month of June, though in the month of January the population remained very low. Highest fungal population was recorded in the month of October in both 1955 and 1965 plantations.

The fungal population in the soil of 1970 plantation decreased in the beginning and the same trend continued from the month of April to July whereafter the population increased and it continued upto the month of October. An abrupt fall in the fungal population was recorded upto the month of January and subsequently the population increased (Fig.1). The least fungal population in this plantation was recorded during the months of June and July. The highest fungal population was recorded in the month of October.

It is observed from the present investigation that effect of different altitudes on the soil fungal population was not remarkable. The fungal population in the soil of both the 1961 (high and low altitudes) plantations exhibited almost a similar pattern (Fig.1). The population decreased from the month of April to June and subsequently increased upto the month of October. Again, the population dropped from the month of November to February and increased thereafter. In both the plantations the least fungal population was recorded in the month of June and the highest figure was obtained in October.

Statistically no significant variations were observed in the soil fungal population between different plantations studied during 1976-1977 but significant variations were observed in the fungal population during different months (Table 1).

Bacterial population in the soil of 1955 and 1965 plantations during 1976-1977 exhibited almost a similar picture. The population in the beginning (i.e. April-May 76) increased in both the plantations and subsequently decreased upto the month of July except in the 1965 plantation where the population increased during the month of July. In both the plantations the bacterial population exhibited a decreasing trend from the month of September to January and thereafter the population increased. In both the plantations the least bacterial population was recorded during the months of November to January. The bacterial population of soil was appreciably high in the month of September, (Fig.2).

In 1970 plantation also the bacterial population increased in the beginning (i.e. April-May) and thereafter a sudden fall in the population was recorded upto the month of July. The population subsequently increased and the peak was noted in the month of September. The population dropped from the month of October and the decreasing trend continued upto the month of January and thereafter it increased. The least bacterial population was recorded in the month of December, (Fig.2).

It was observed that there was not much effect of different altitudes on the soil bacterial population as the population exhibited a similar trend in both 1961 plantations on high and low altitudes (Fig.2). A low count was obtained in the months April-July.and October-January. The peak population was recorded in August (Fig.2). Statistically there was no significant variations in the bacterial population of different plantations, significant variation was, however, noted in the bacterial population of different months (Table 2).

Actinomycetes population of the soil in all the plantations exhibited almost a similar pattern of variation. The peak was recorded in the month of September in all the plantations (Fig.3). The population dropped during the months of June and July in all the plantations except in 1965 plantation where an increase was recorded. In all the plantations there was an increasing trend in the actinomycetes population from the months of February - March, though fairly low count was obtained during the months of November - January.

Statistically no significant variation in the soil actinomycetes population was observed between different plantations, but significant variation was observed in different months (Table - 3).

The fungal population of the soil of 1955 and 1970 pine plantations studied during 1977-1978 exhibited almost a similar picture (Fiv.4). Surprisingly there was not much variation in the quantity of soil fungal population in these two plantations during the course of investigation (Figs. 1 and 4). It was observed that the fungal population in the soil of both the plantations showed an increasing trend from the beginning (i.e. from the month of June to September) and a sudden fall in the population was recorded in the month of October and thereafter the population increased upto the month of November and it dropped again till January. An increase in the population was marked again upto the month of February whereafter it remained almost same. The least fungal population was recorded in the month of October and peak was noted in the month of September 1977 (Fig.4).

During 1977-1978 statistically a significant variation was observed in the fungal population of soil between two different pine plantations unlike that of 1976-1977. A significant variation in the population was also recorded between different sampling periods (Table 4).

The bacterial and actinomycetes population in the soil of 1955 and 1970 pine plantations during 1977-1978 exhibited a similar trend (Fig.5), but a substantial

increase in the population of bacteria and actinomycetes was observed during the second year of studies (Fig.5).

The population of bacteria and actinomycetes which was low in the beginning (i.e. from the months of May - June) in the soil of both the plantations abruptly increased in the month of August. Thereafter, the population of bacteria and actinomycetes in both the plantations declined and the least population was attained in the month of January 1978 (Fig.5). An increase was again recorded after this fall.

Like 1976-1977, statistically no significant variation in the population of bacteria and actinomycetes was recorded between the two plantations during 1977-1978 also but a significant variation in the population was recorded during the different periods of sampling (Tables 5-6).

2. Percentage relative abundance and frequency of fungal species under different pine plantations during 1976-1978 :-

Sixteen fungal species, viz. 6 phycomycetes, 1 ascomycetes (unidentified), 1 sterile mycelia and 8 members of fungi imperfecti were isolated from the soil of 1955 plantation during 1976-1977 (Table 7) where as only eight species were isolated from the same pine stand during second year i.e. 1977-1978 (Table 17).

Absidia cylindrospora, *Pythium* sp and *Trichoderma viride* were isolated with high frequency and with fairly high population than any other fungal species during 1976-1977. *Pythium* showed maximum population (according to percentage relative abundance) in the soil at different samplings (Fig.6). However, *Trichoderma viride* was recorded as dominant fungus according to percentage frequency

(Table 7). Other fungal species recorded occured sporadically. Similarly Absidia cylindrospora, Circinella sp, Pythium sp, Phoma humicola and Trichoderma viride were isolated regularly with high population in 1977-1978. Pythium sp and Trichoderma viride exhibited the maximum population at different samplings (Fig.11). Absidia cylindrospora and Trichoderma viride were, however, recorded as dominant fungi according to percentage frequency (Table 17). Other fungal species occured sporadically. Statistically significant variation was observed in the percentage relative abundance between different fungal species isolated during 1976-1978 (Table 12, 19).

Fifteen fungal species, viz. 5 phycomycetes, 1 ascomycetes, 1 sterile mycelia and 8 members of fungi imperfecti were isolated from the soil of 1965 plantation (Table 8). Absidia cylindrospora, Pythium sp, Trichoderma viride and Penicillium chrysogenum were observed with high frequency and with high population at different samplings (Fig.7). However, Pythium sp was maximum in the soil (Fig.7). According to the frequency of isolation, Pythium sp, Penicillium chrysogenum and Trichoderma viride were dominant fungi while the other fungal species isolated occured sporadically (Table 8). Statistically significant variation was observed in the percentage relative abundance between different fungal species isolated (Table 13).

Fourteen fungal species, viz., 4 phycomycetes, 1 ascomycetes, 1 sterile mycelia, 1 unidentified fungus and 7 fungi imperfecti were isolated from 1970 plantation during

1976-1977 (Table 9) where as the fungal species isolated from the same plantation during 1977-1978 were thirteen (Table 18). *Absidia cylindrospora*, *Pythium* sp, *Trichoderma viride* and *Penicillium chrysogenum* were frequently isolated with fairly high population throughout 1976-1978 (Figs.8 &12). *Phoma humicola* which was frequently isolated during 1977-1978 with high population (Fig.12) was recorded in the previous year with less population (Fig.8). *Absidia cylindrospora* and *Penicillium chrysogenum* were dominant soil fungi in first year (Table 9). In second year *Absidia cylindrospora* *Pythium* sp, *Phoma humicola* and *Trichoderma viride* were dominant species of this plantation (Table 18). Other species occured sporadically.Statistically significant variation was observed in the percentage relative abundance between different fungal species isolated during 1976-1978 (Table 14 and 20).

Almost similar forms of fungi were isolated from the soil of 1961 plantations raised at high and low altitudes (Tables 10,11). *Absidia cylindrospora*, *Pythium* sp, *Trichoderma viride* and *Penicillium chrysogenum* were noted with high frequency and with relatively higher population from both the stands (Fig. 9,10). *Pythium* sp was isolated with maximum population at different samplings from both the plantations. *Trichoderma viride* was dominant fungus in both the stands (Table 10,11) whereas *Absidia cylindrospora* and *Pythium* sp were dominant in soil of high altitude only (Table 10).

Statistically significant variation was observed in the percentage relative abundance between different fungal

species isolated from both the plantations (Tables 15,16).

3. Seasonal distribution of soil fungi of different groups in pine plantations during 1976-1978 :-

Phycomycetes - This class was represented by 8 genera (Tables 21-23). Most of the genera were distributed in all the three seasons (i.e. summer, rainy and winter). Their distribution, however, was not similar in all the plantations during 1976-1977. The distribution of Pythium sp and Absidia cylindrospora during rainy and winter seasons was very high according to their percentage frequency (Tables 21-23). Mucor hiemalis was almost absent during summer season except in the soil of 1970 plantation. Cunninghamella echinulata was recorded appreciably in high percentage during rainy season but it was altogether absent from the soil of all the plantations during the winter season (Tables 21-23), in 1976-1977.

During second year of investigation the phycomycetes was represented by six genera (Table 24) out of which only two species Absidia cylindrospora and Pythium sp were distributed with 100 % frequency during the summer and rainy seasons in both 1955 and 1970 pine plantations. Another fungus of this class Circinella sp was distributed in the soil of 1955 pine plantation during all the three seasons in 1977-1978 with fairly high frequency, but it was absent from 1970 plantation. Other three species like Mucor hiemalis, Actinomucor sp and Cunninghamella echinulata were isolated but they differed in their frequency distribution. They were isolated with very low frequency during 1977-1978.

Ascomycetes - This class of fungi were very rare in the soil of pine forest of Meghalaya. Some members of this class were isolated occasionally but their relative abundance and percentage frequency were low. Only two genera, viz., Thielavia sp and Chaetomium globosum were sometimes isolated and that too they were restricted in the soil of only two to three pine plantations (Tables 21-23). Another unidentified ascomycetes was isolated from the soil of 1961 pine plantation on high altitude with only 33.3 % frequency.

Thielavia sp was isolated from the soil of 1961 pine plantation on high altitude only during summer and it was altogether absent from other plantations during this season. Chaetomium globosum was isolated from the soil of 1965 and 1970 plantations with only 25 % frequency during rainy season and it was absent during other seasons (Tables 21-23). Though some of the fungal species of this class were isolated from the soil during 1977-1978, they were not equally distributed in all the plantations in the different seasons. Only four species, viz., Thielavia sp, Chaetomium globosum, Gelasinospora sp and Sordaria sp with low percentage frequency were cultured (Table 24).

Deuteromycetes - This class was represented by nine genera (Tables 21-23) out of which Trichoderma viride and Penicillium chrysogenum were noted with high frequency in all the three seasons in 1976-1977. Another fungus Phoma humicola was also recorded from the soil of almost all the plantations during different seasons with fairly high percentage frequency (Tables 21-23).

During 1977-1978 the class deuteromycetes was represented by only four species amongst which Trichoderma viride was isolated from the soil of both 1955 and 1970 plantations with 100 % frequency during all the three seasons. Phoma humicola was isolated from the soil of both 1955 and 1970 plantations during all the three seasons with high percentage frequency. Penicillium chrysogenum was isolated from the soil of 1970 plantation during all the three seasons with high percentage frequency but it was totally absent from the soil of 1955 plantation (Table 24). A sterile white mycelia was isolated with 50 % frequency in the soil of 1970 plantation during summer. It could not, however, be isolated from the soil of 1955 plantation during 1977-1978.

4. Moisture content and pH of the soil during 1976-1978 :-

The moisture content of the soil in all the plantations was sufficiently high during the months of May-December (Tables 25 and 27) and thereafter the value was low. Statistically moisture content of the soil varied significantly between plantations and also between different sampling periods during 1976-1977, (Table 26). There was, however, no significant variation in the moisture content of the soil between 1955 and 1970 pine plantations during 1977-1978. The moisture content varied significantly between different sampling periods (Table 28).

The average pH value of the soil in all the plantations was above 6.00 during 1976-1977 except in 1965 plantation where this value was 5.88 (Table 29). Statistically no significant variation was obtained in the pH of the soil

between plantations and between different sampling periods (Table 30) during 1976-1977.

Almost similar result was obtained for the pH of the soil during 1977-1978. The average pH value of the soil of 1955 plantation was observed to be above 6.00 in 1970 plantation (Table 31).

Statistically no significant variation was obtained in the pH of the soil between plantations but significant variations were obtained between sampling periods during 1977-1978 (Table 32).

5. <u>Relationship between Co_2 evolution, dehydrogenase activities, total microbial population and other physico-chemical properties of soil of 1970 pine plantation studied during 1977-1978</u> :-

Statistically no correlation between dehydrogenase activities and total microbial population of the soil was observed, (Fig.14 and Table 33).

There was an abrupt fall in the dehydrogenase activities with an increase in the total microbial population in the month of August thereafter a fall in the total microbes was observed, and this fall in the population continued upto the month of November but the corresponding dehydrogenase activities of the soil during the period gradually increased (Fig.14). The addition of glucose to the soil as substrate substantially increased the dehydrogenase activities of the soil (Fig.14), and the values for the simultaneous measurements of dehydrogenase activities of soil with glucose and without glucose was found to be highly correlated ($r = 0.949$, $P < 0.001$), (Table 33).

From this experiment statistically a positive

correlation could be observed between carbon-dioxide evolution and total microbial population of the soil since the values for the simultaneous measurement of Co_2 production and total microbial population was found to be highly correlated ($r = 0.732$, $P < 0.01$), (Table 33). With an increase in the total microbial population corresponding increase in the Co_2 evolution from the soil was observed during the months of July-August and again when total microbial population of the soil declined during the months of September to January there was a corresponding fall in the evolution of Co_2 from the soil (Fig.14). The peak in population was recorded in the month of August when the Co_2 evolution from the soil was also maximum (Fig.14).

Statistically no correlation could be established between dehydrogenase activities and Co_2 evolution from the soil (Fig.15) and (Table 33).

From the present investigation, the dehydrogenase activities of the soil seemed to be influenced more profoundly by moisture content of the soil as the values of the measurements of the dehydrogenase activities and moisture content of the soil was found to be negatively correlated ($r = -0.764$, $P < 0.01$) (Table 33). The effect of moisture content on Co_2 evolution rates were apparent (Fig.15) but no statistical correlation was observed between the parameters (Table 33). The pH of the soil did not influence Co_2 evolution in this forest, though there was significant difference in the soil pH during different seasons, but it influenced the dehydrogenase activities of

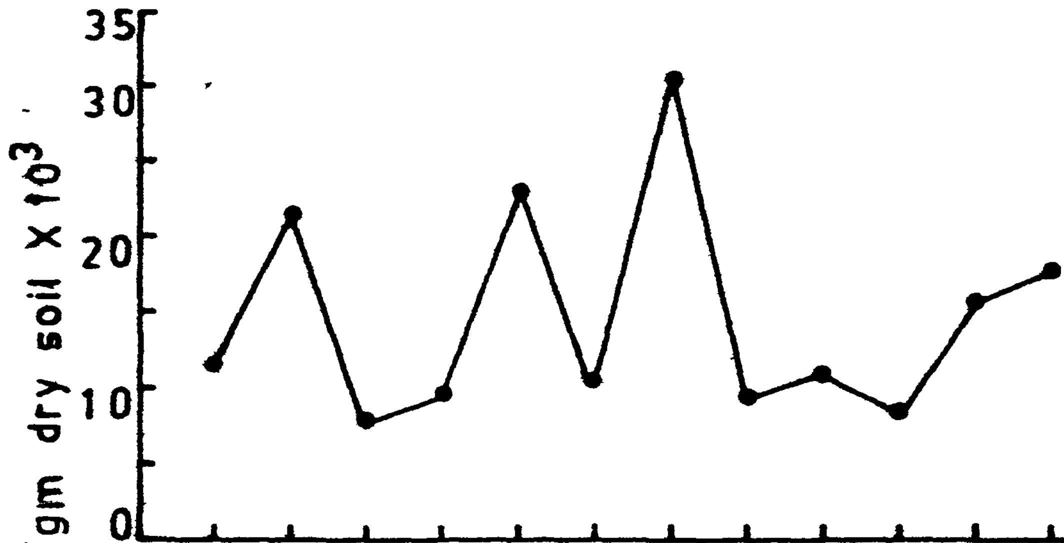
the soil.

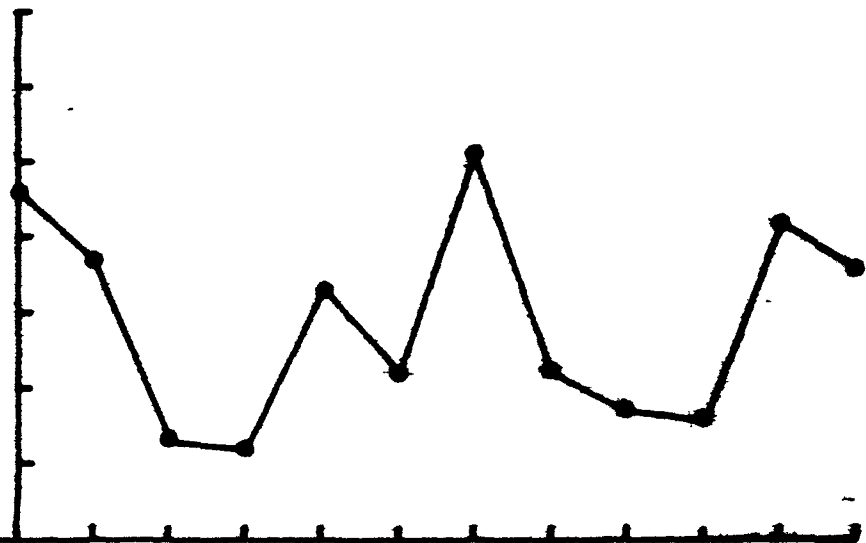
Statistically a significant correlation was observed between the soil pH and dehydrogenase activities as the values for the simultaneous measurement of soil pH and dehydrogenase activities were found to be significantly correlated ($r = 0.696$, $P < .05$), (Table 33).

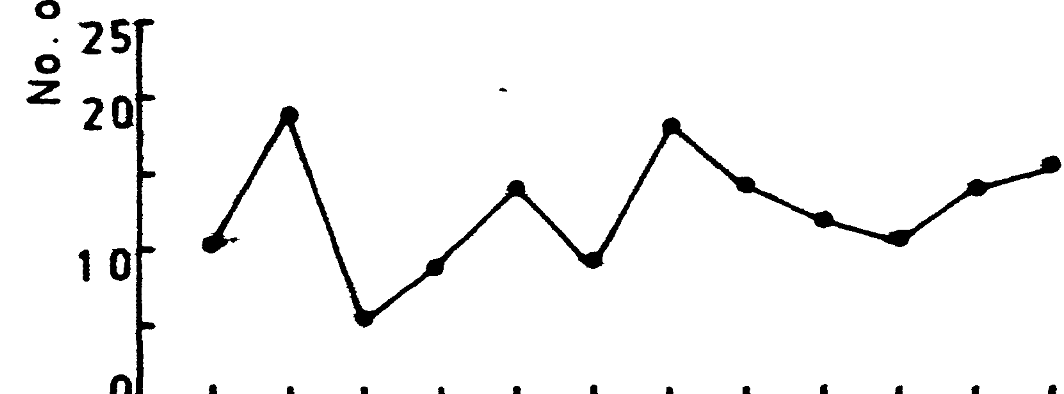
Organic matter content of the soil did not influence Co_2 evolution, dehydrogenase activities and microbial population of the soil (Fig.15) and no correlation was found between the parameters (Table 33).

Soil nitrogen did not influence Co_2 evolution, dehydrogenase activities and total number of microbes. No correlation was observed between these parameters. (Table 33).

Figure 1 : Seasonal variation in fungal population of soil in different pine plantations studied during 1976 - 1977.







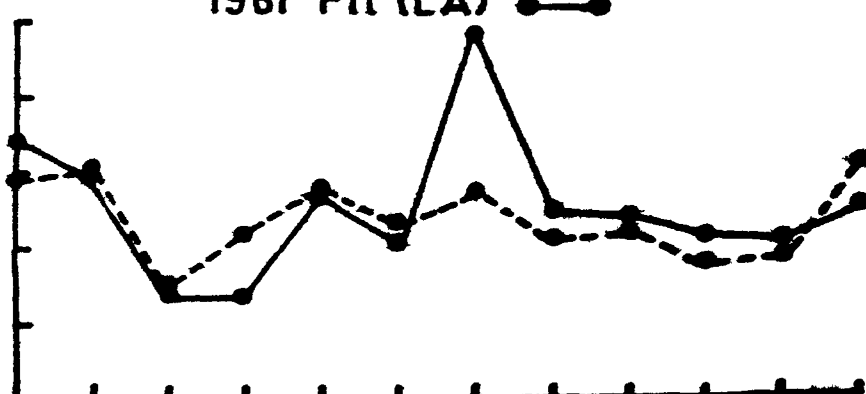
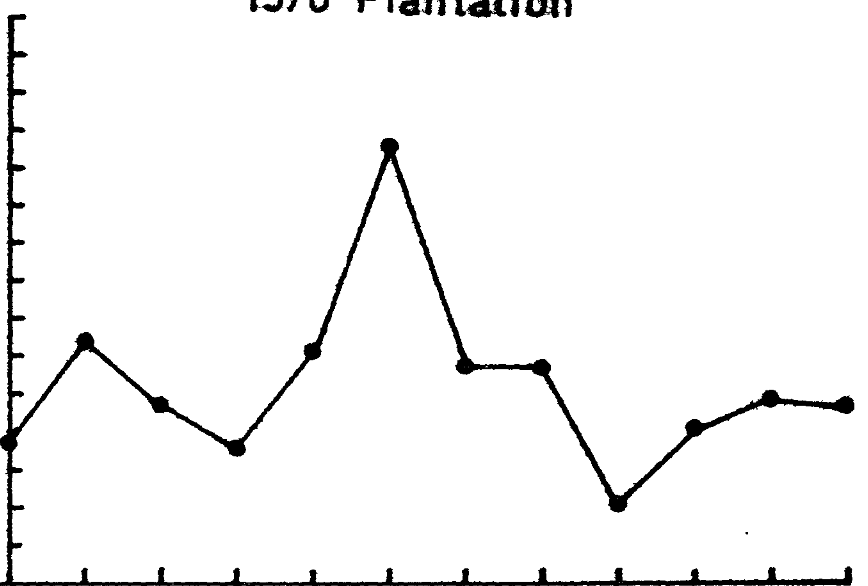
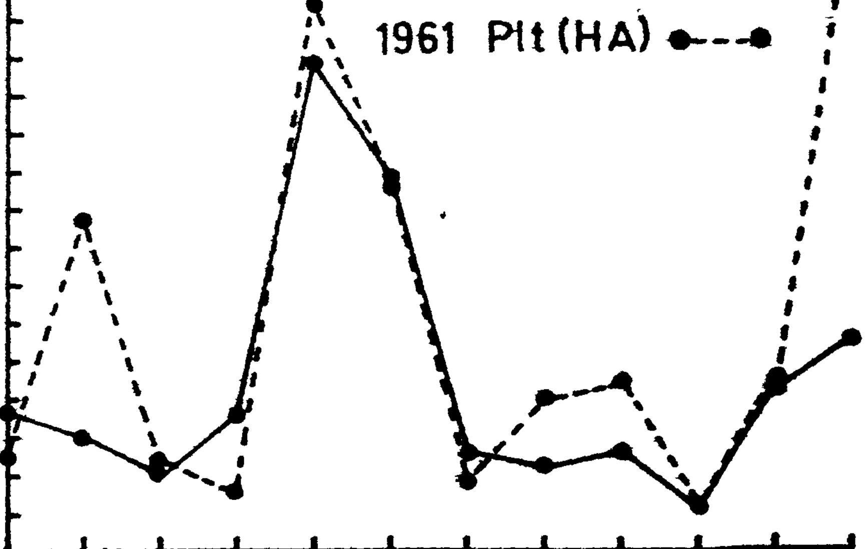


Figure 1

Figure - 2. Seasonal variation in bacterial population of soil in different pine plantations studied during 1976-1977.





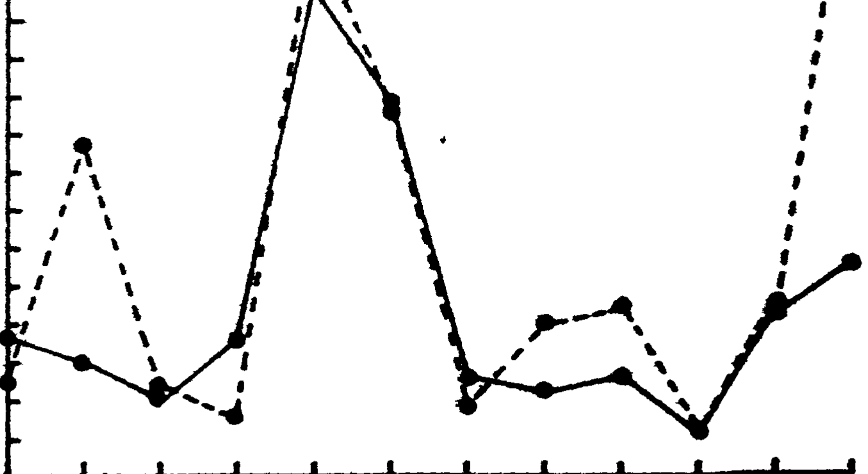
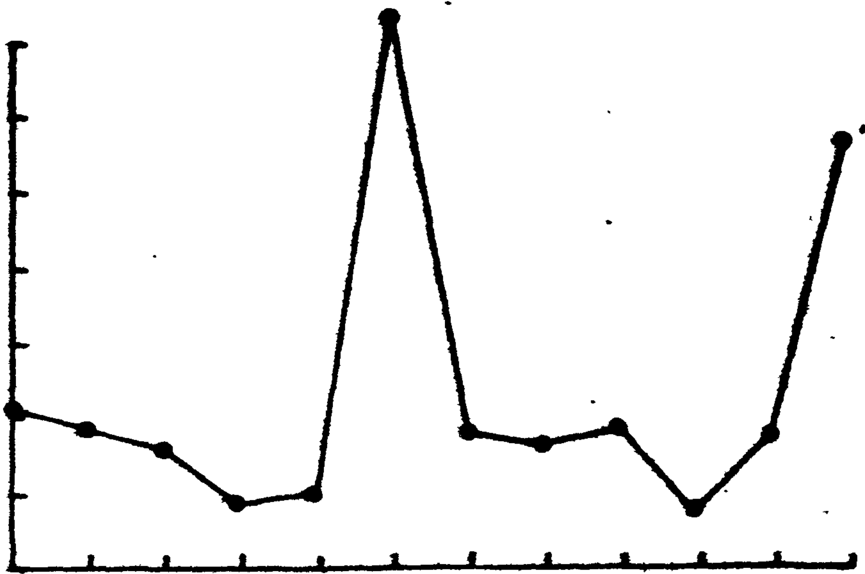
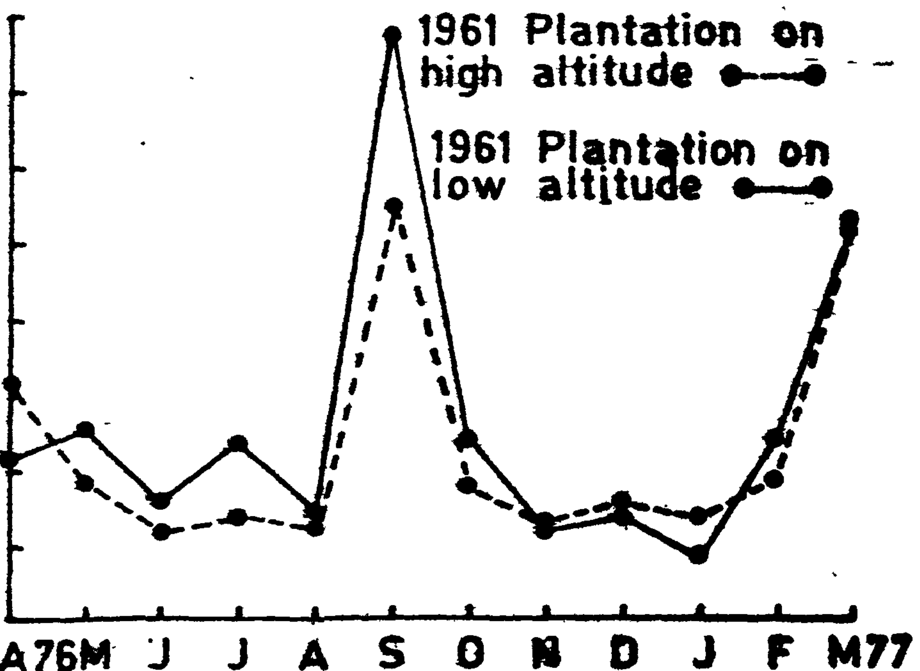
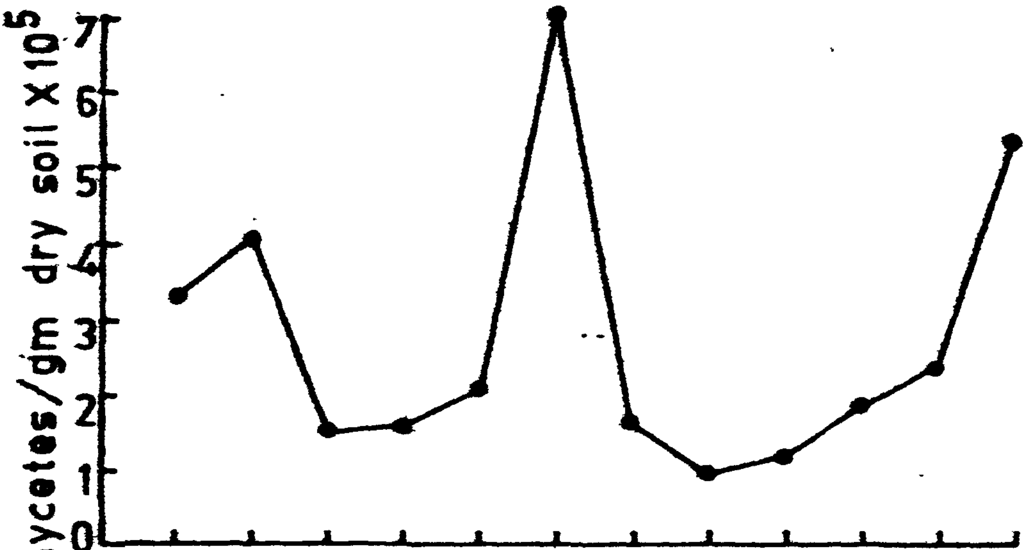


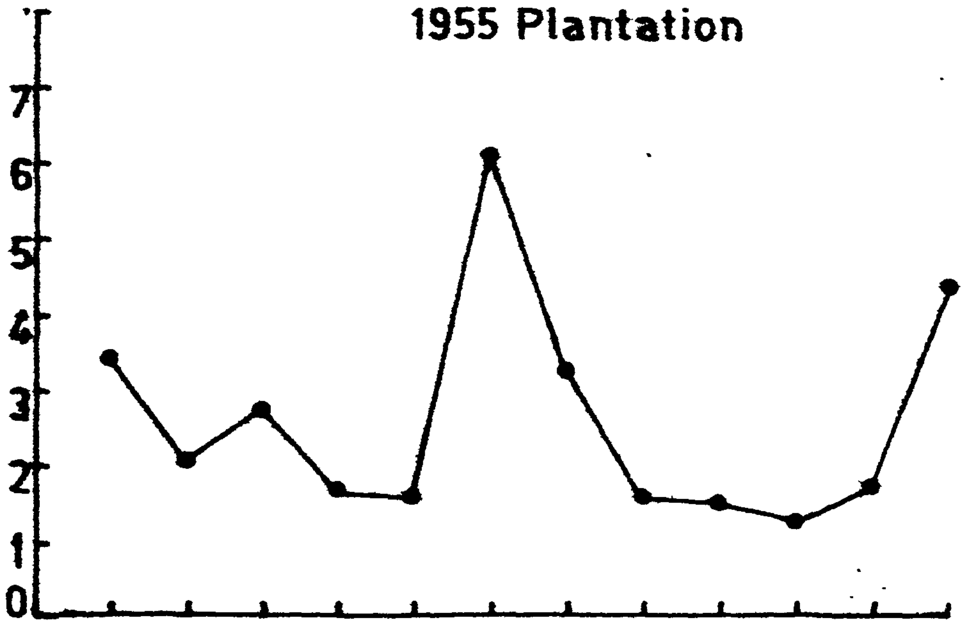
Figure 2

Figure - 3 : Seasonal variation in actinomycetes population of soil in different pine plantations studied during 1976-1977.









SAMPLING PERIODS

Figure 3

Figure - 4 : Seasonal variation in fungal population of soil of two pine plantations studied during 1977-1978.

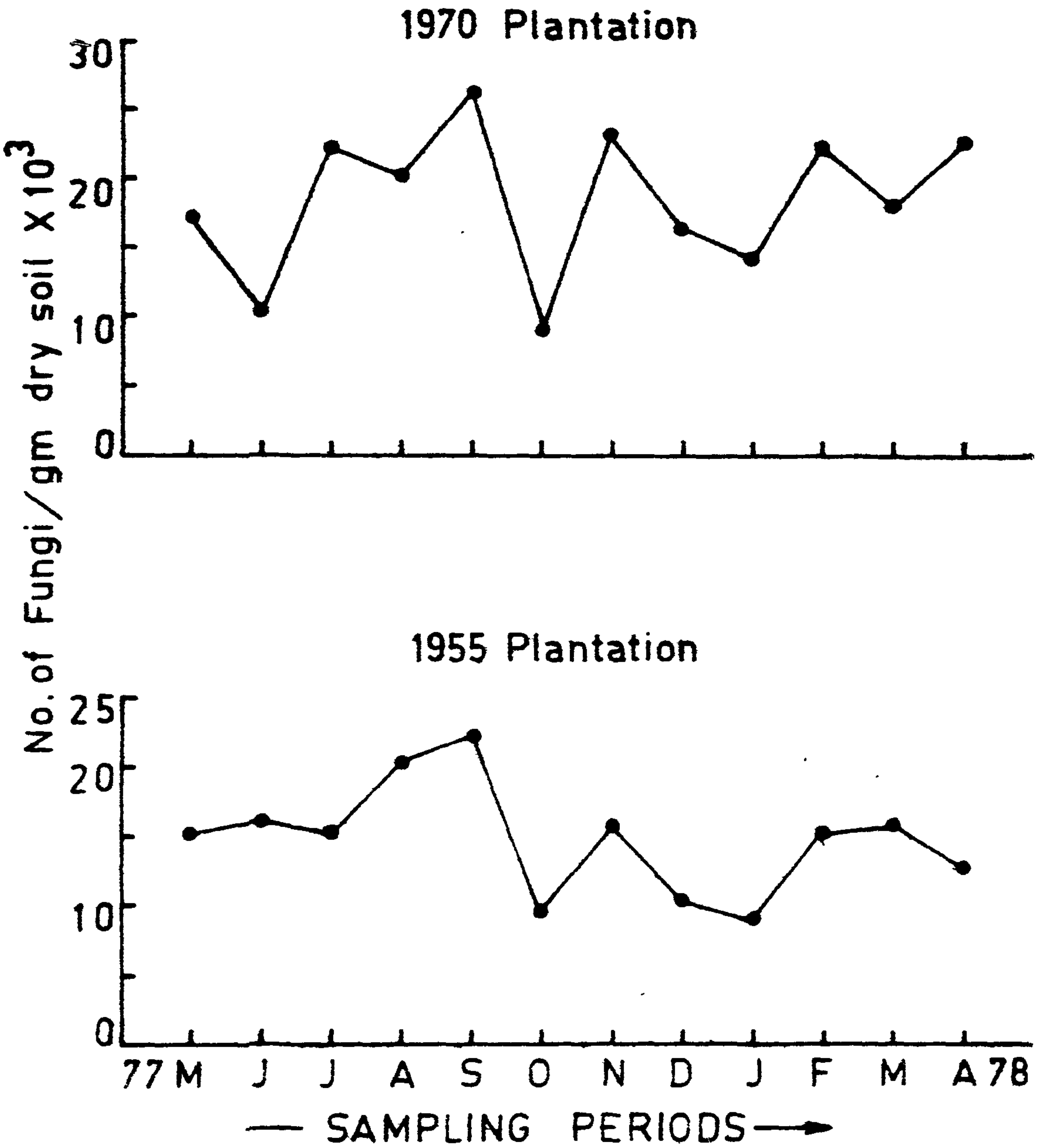


Figure 4

Figure - 5 : Seasonal variation in bacterial and actinomycetes population of soil of two pine plantations studied during 1977-1978.

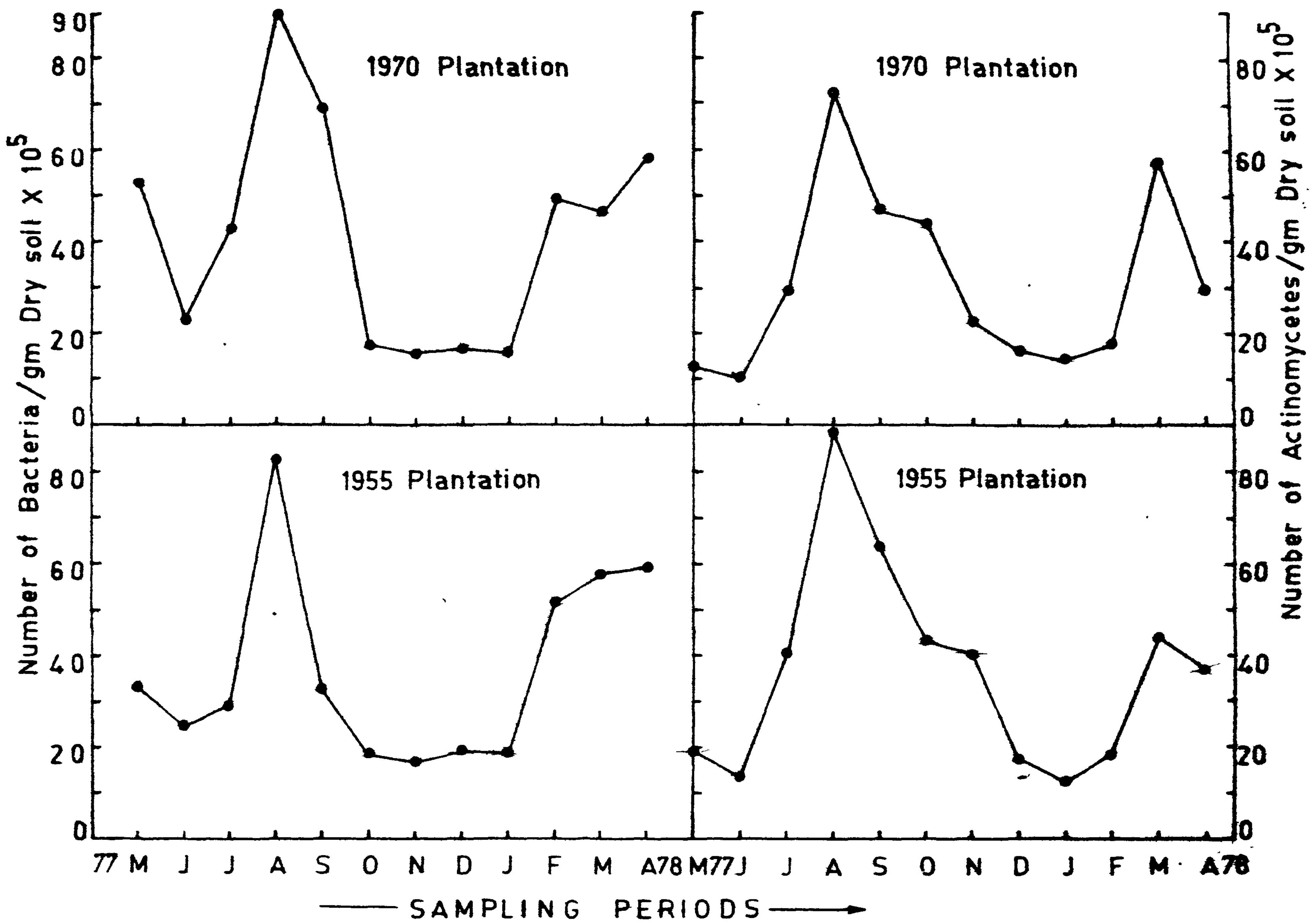


Figure 5

Figure - 6: Seasonal variation in percentage relative abundance of various fungal species, isolated from the soil of 1955 pine plantation during 1976-1977.

1.= *Absidia cylindrospora*
2 = *Circinella* sp, 3 = *Mucor hiemalis*,
4 = *Cunninghamella echinulata*,
5 = *Pythium* sp., 6 = *Phoma humicola*,
7 = *Trichoderma viride*, 8 = *Aspergillus niger*, 9 = *Penicillium chrysogenum*,
10 = *Gliocladium roseum*, 11 = *Verticillium* sp, 12 = *Cladosporium herbarum*,
13 = *Fusarium sporotrichoides*,
14 = Sterile white mycelia.

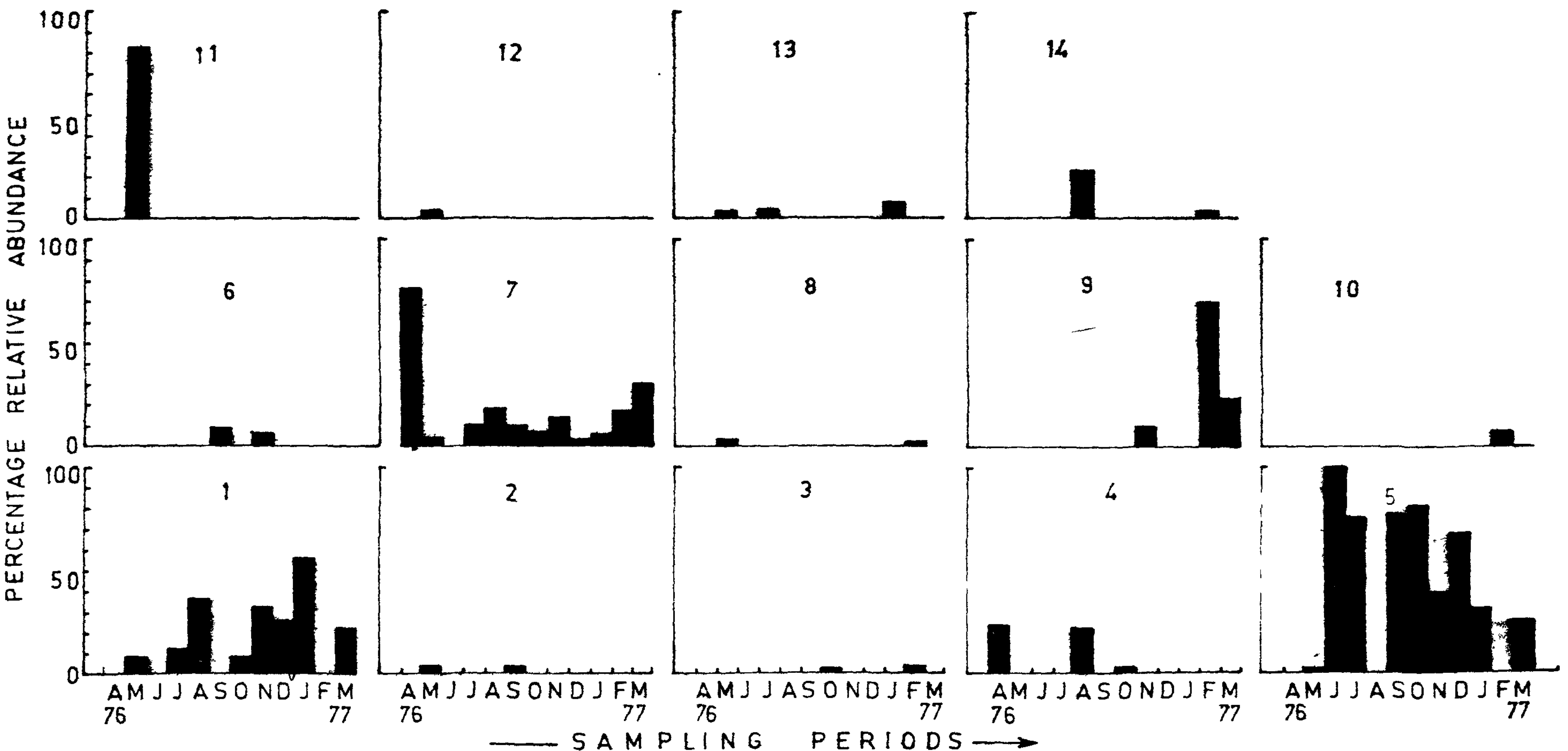


Figure 6

Figure - 7: Seasonal variation in percentage relative abundance of various fungal species, isolated from the soil of 1965 pine plantation during 1976-1977. 1 = *Absidia cylindrospora*, 2 = *Mucor hiemalis*, 3 = *Zygorhynchus* sp. 4 = *Cunninghamella echinulata*, 5 = *Pythium* sp. 6 = *Chaetomium globosum*, 7 = *Phoma humicola*, 8 = *Trichoderma viride* 9 = *Penicillium chrysogenum*, 10 = *Scopulariopsis* sp., 11 = *Gliocladium roseum*, 12 = *Verticillium* sp., 13 = *Alternaria tennuis*, 14 = *Fusarium sprotrichoides*, 15 = Sterile white mycelia.

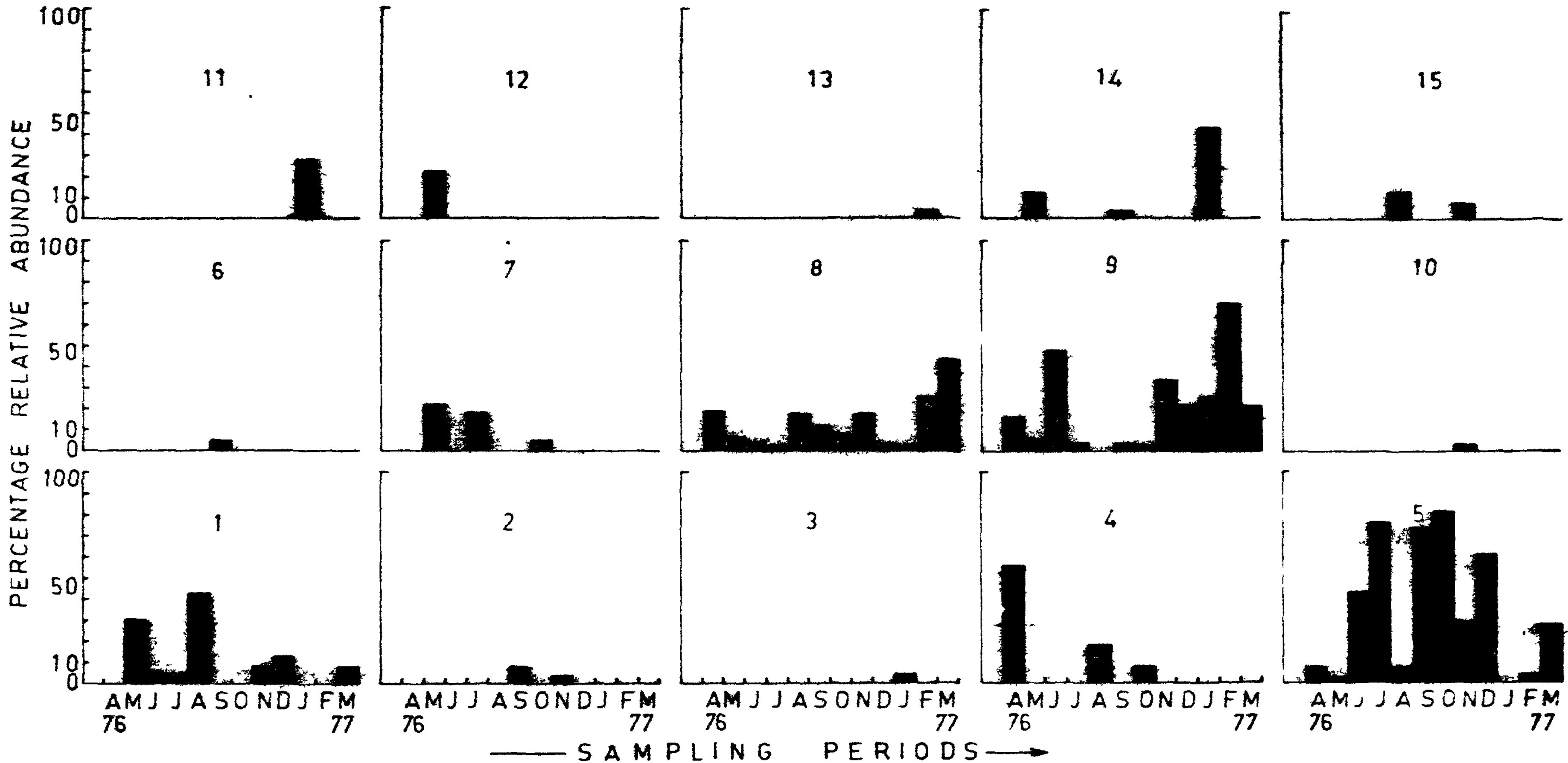


Figure 7

Figure - 8 : Seasonal variation in percentage relative abundance of various fungal species, isolated from the soil of 1970 pine plantation during 1976-1977.
1 = *Absidia cylindrospora*,
2 = *Circinella* sp., 3 = *Mucor hiemalis*,
4 = *Pythium* sp., 5 = *Chaetomium globosum*, 6 = *Phoma humicola*, 7 = *Trichoderma viride*, 8 = *Aspergillus niger*
9 = *Penicillium chrysogenum*,
10 = *Verticillium* sp., 11 = *Cladosporium herbarum*, 12 = *Fusarium sporotrichoides*,
13 = Sterile white mycelia.

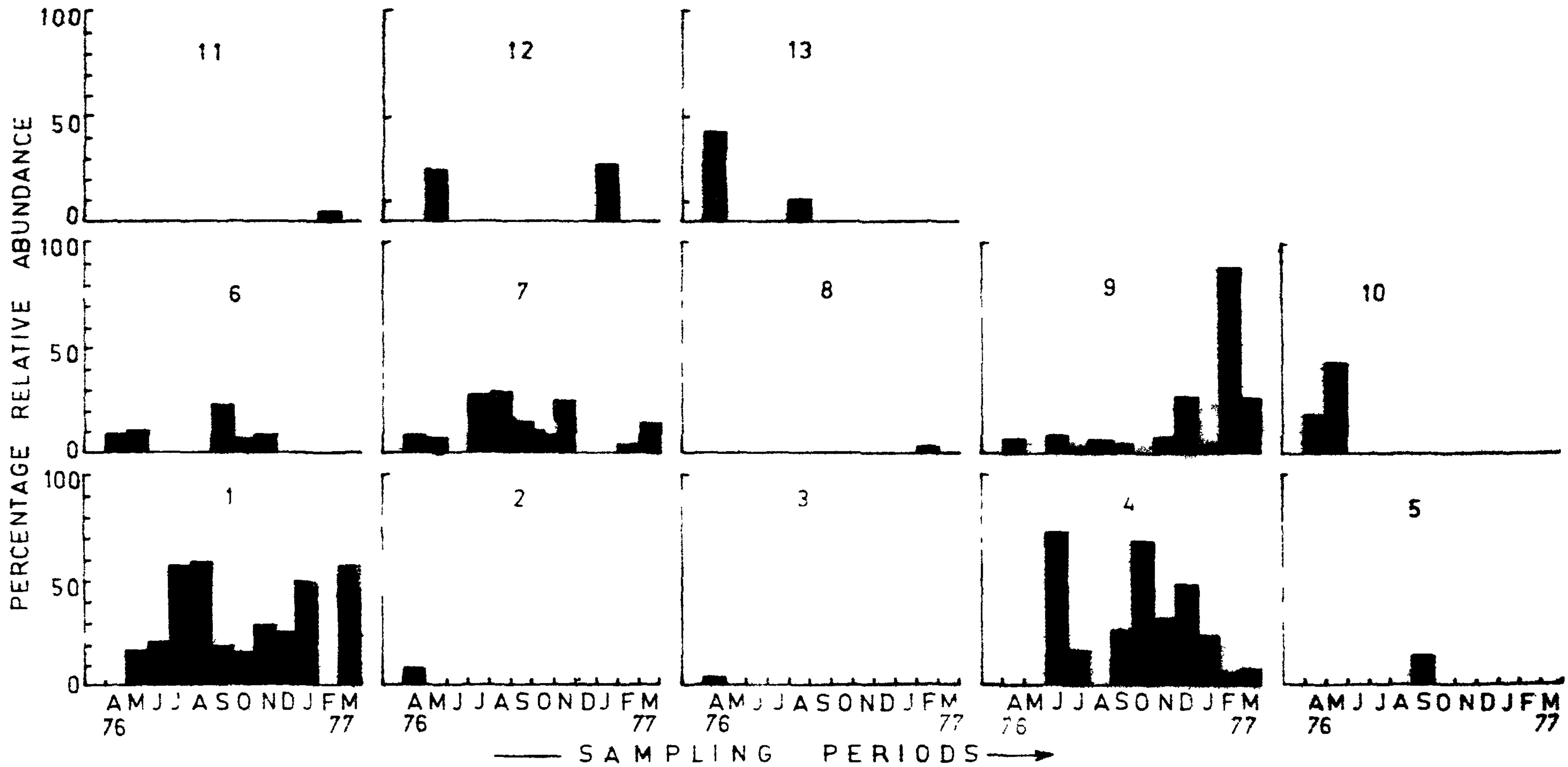


Figure 8

Figure - 9 : Seasonal variation in percentage relative abundance of various fungal species, isolated from the soil of 1961 (high altitude) pine plantation during 1976-1977.

1 = *Absidia cylindrospora*, 2 = *Rhizopus nigricans*, 3 = *Cunninghamella echinulata*, 4 = *Pythium* sp., 5 = *Thielavia* sp., 6 = *Phoma humicola*, 7 = *Trichoderma viride*, 8 = *Aspergillus niger*, 9 = *Penicillium chrysogenum*, 10 = *Verticillium* sp., 11 = *Fusarium sporotrichoides*, 12 = Sterile white mycelia.

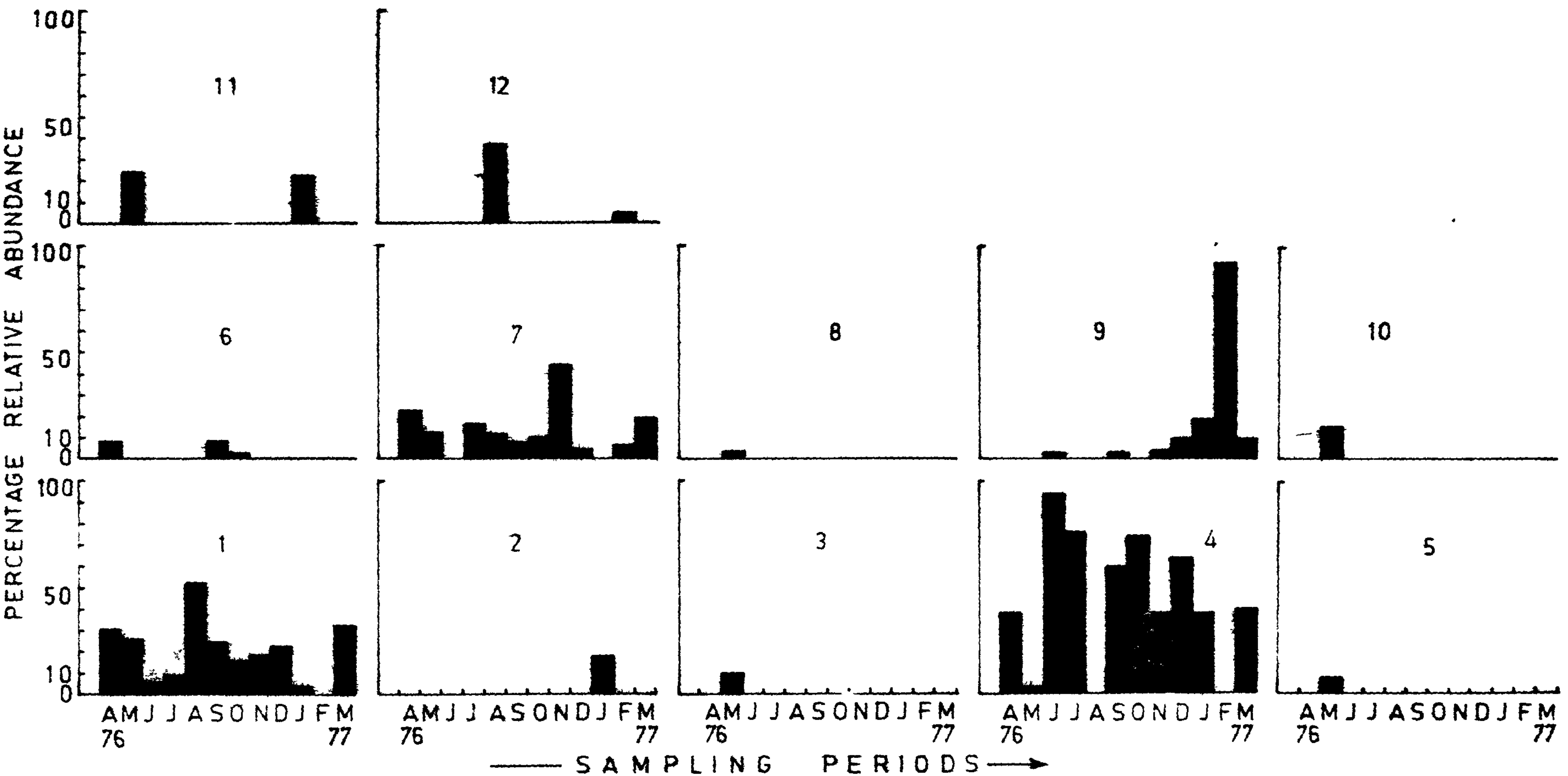


Figure 9

Figure - 10 : Seasonal variation in percentage relative abundance of various fungal species, isolated from the soil of 1961 (Low altitude) pine plantation during 1976-1977.

1 = *Absidia cylindrospora*,
2 = *Rhizopus nigricans*, 3 = *Circinella* sp., 4 = *Mucor hiemalis*,
5 = *Cunninghamella echinulata*,
6 = *Pythium* sp., 7 = *Phoma humicola*,
8 = *Trichoderma viride*, 9 = *Aspergillus niger*, 10 = *Penicillium chrysogenum*, 11 = *Verticillium* sp.,
12 = *Cladosporium herbarum*,
13 = *Fusarium sporotrichioides*,
14 = Sterile white mycelia.

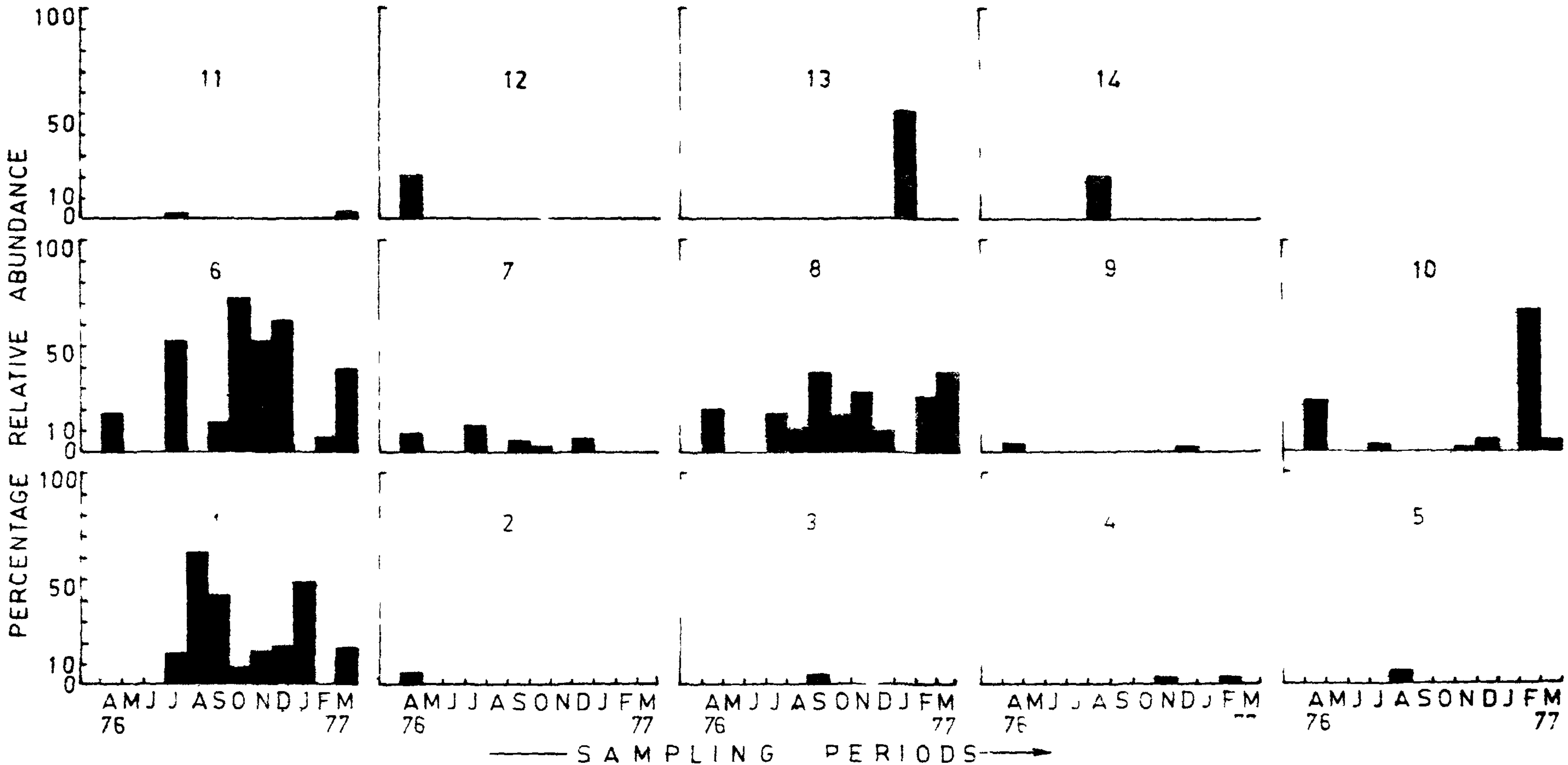


Figure 10

Figure - 11: Seasonal variation in percentage relative abundnace of various fungal species, isolated from the soil of 1955 pine plantation during 1977-1978.

1 = *Absidia cylindrospora*, 2 = *Circinella* sp., 3 = *Cunninghamella echinulata*, 4 = *Pythium* sp., 5 = *Gelasinospora* sp., 6 = *Sordaria* sp. 7 = *Phoma humicola*, 8 = *Trichoderma viride*.

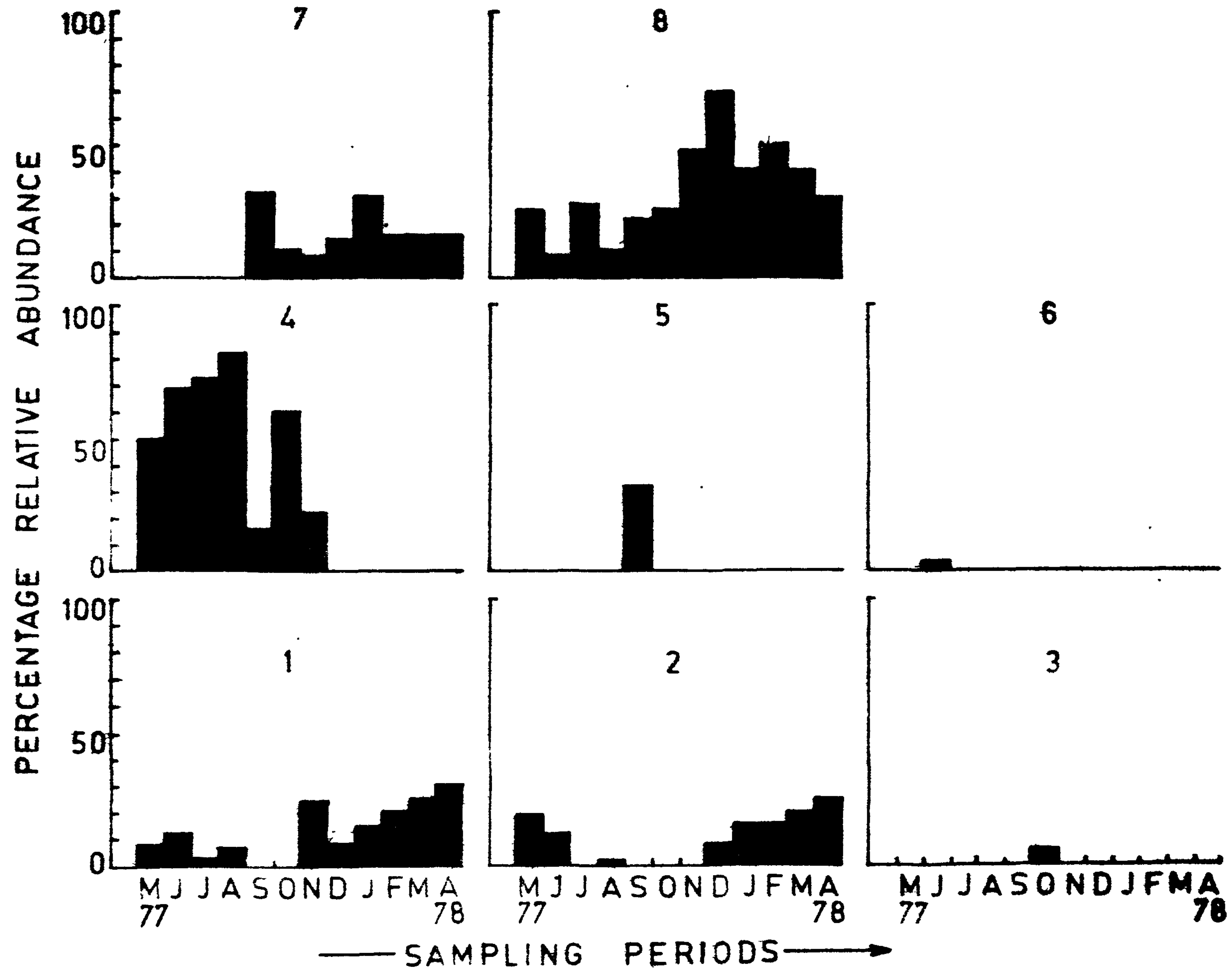


Figure 11

Figure - 12 : Seasonal variation in percentage relative abundance of various fungal species, isolated from the soil of 1970 pine plantation during 1977-1978.
1 = *Absidia cylindrospora*, 2 = *Mucor hiemalis*, 3 = *Actinomucor* sp.,
4 = *Cunninghamella echinulata*
5 = *Pythium* sp., 6 = *Thielavia* sp.
7 = *Chaetomium globosum*, 8 = *Gelasinospora* sp., 9 = *Phoma humicola*,
10 = *Trichoderma viride*, 11 = *Penicillium chrysogenum*, 12 = *Gliocladium roseum*.

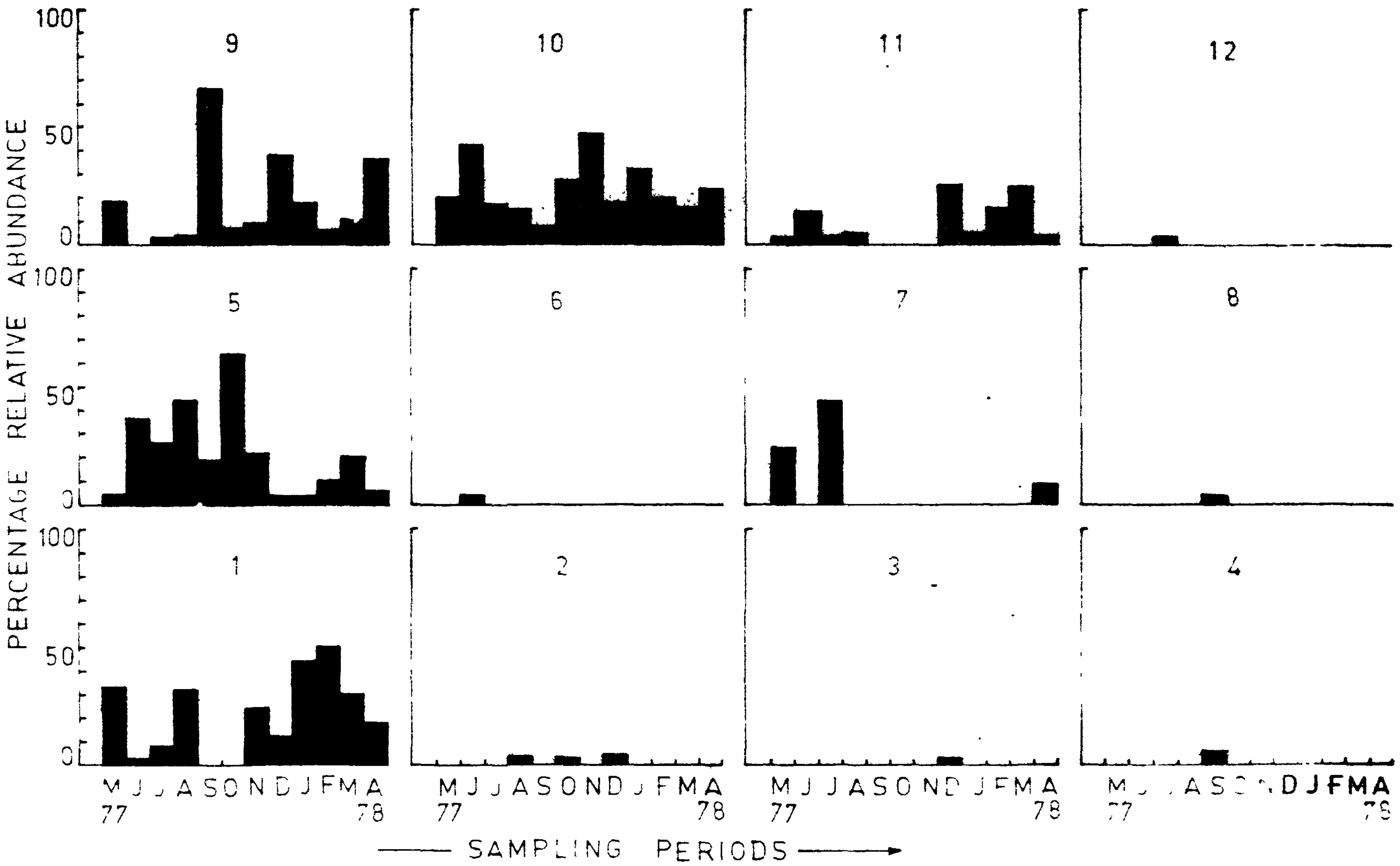


Figure 12

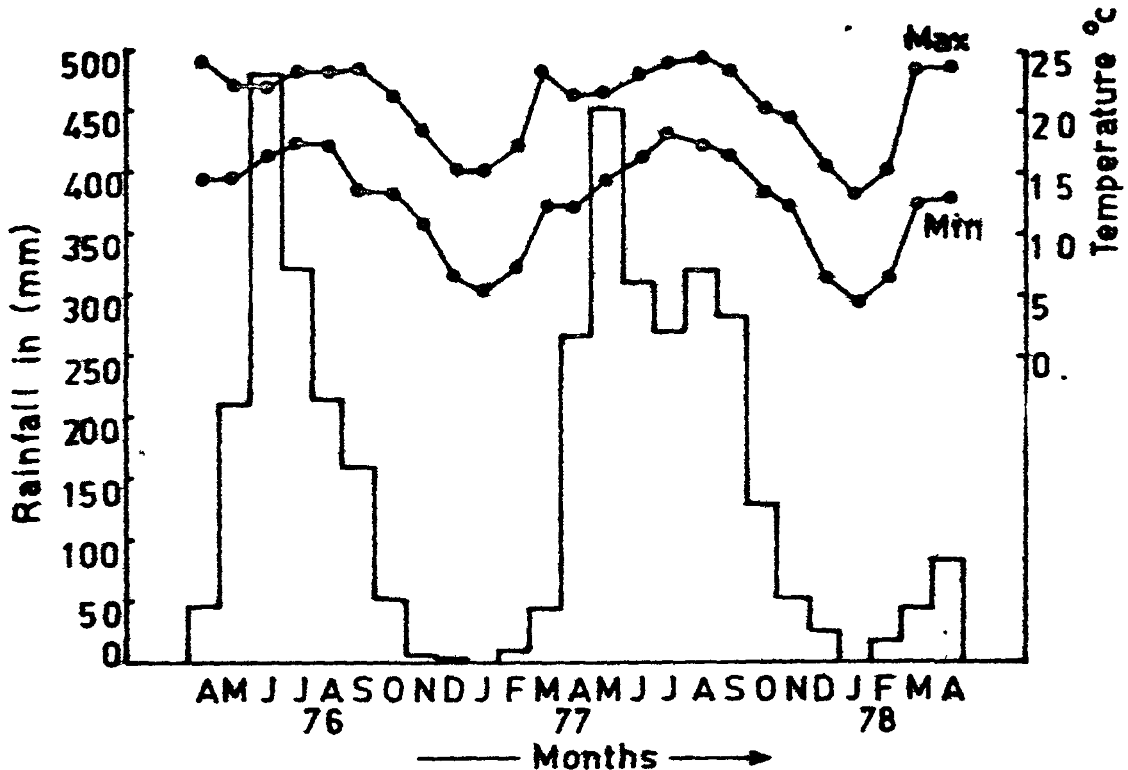


Fig.13 Monthly climatic data during 76-78

Figure - 14 : Relationship between total number of microbes, carbon dioxide evolution and dehydrogenase activity of the soil of 1970 plantation studied during 1977-1978.

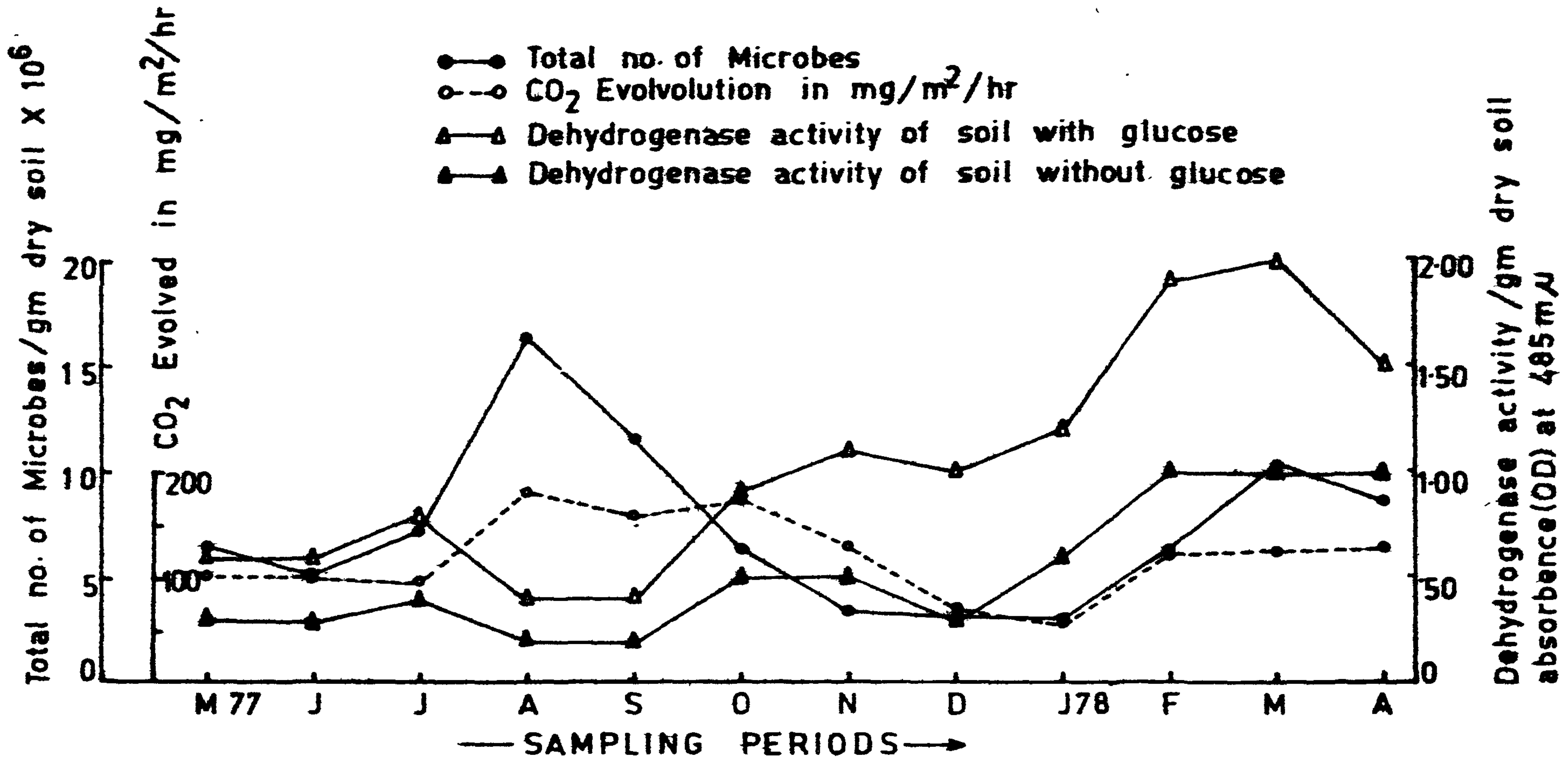


Figure 14

Figure - 15 : Relationship between organic matter content, carbondioxide evolution, dehydrogenase activity and moisture content of the soil of 1970 plantation studied during 1977-1978.

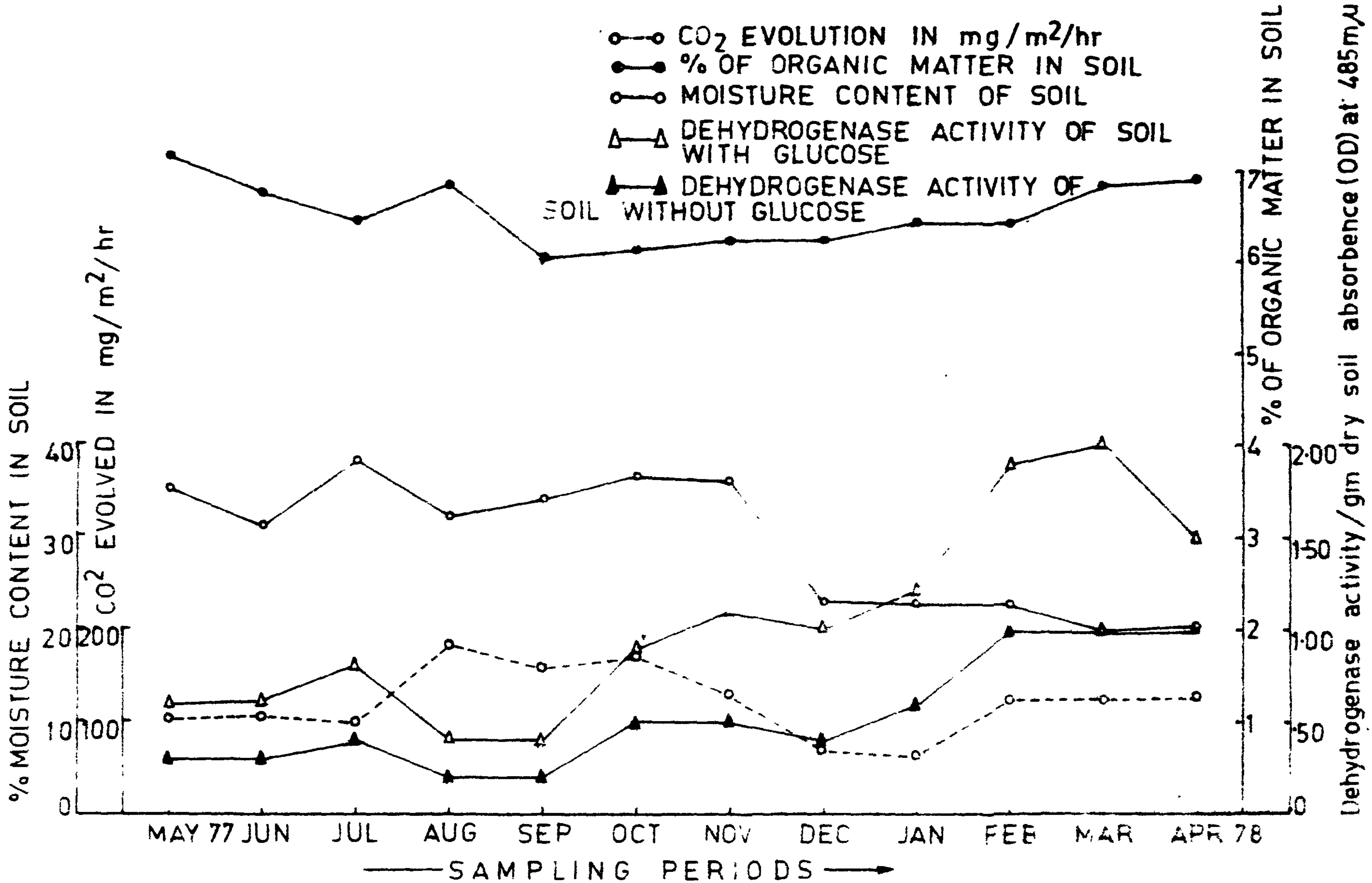


Figure 15

Table - 1 Analysis of variance for the fungal population (No.of Fungi/gm dry soil x 10^3) of the soil of different pine plantations for April 76 - March 77.

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value 5 %	Remarks
Between plantations	4	7237.58	1809.40	1.204	2.592	Not significant
Between sampling periods	11	109263.99	9933.09	6.607	2.029	Significant
Error	44	66143.28	1503.25			
Total	59					

The above table reveals that the variation in the fungal population of the soil between different Pine plantations is not significant but it is significant between different sampling periods.

Table - 2. Analysis of variance for the bacterial population (No. of bact/gm. dry soil x 10^5) of the soil of different pine plantations for April 1976 - March 1977.

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' 5 %	Remarks
Between plantations	4	162159.71	40539.93	0.695	2.592	Not significant
Between sampling periods	11	4363824.76	396711.34	6.802	2.029	Significant
Error	44	2566184.62	58322.38			
Total	59					

The above table reveals that the variation in the bacterial population of the soil between different Pine plantations is not significant but it is significant between different sampling periods.

Table - 3. Analysis of variance for the actinomycetes population (No. of act/gm dry soil x 10^5) of the soil of different pine plantations for April 76 - March 77.

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value 5 %	Remarks
Plantations	4	5611.77	1402.94	0.388	2.592	Not significant
Sampling periods	11	1591926.80	144720.62	40.06	2.029	Significant
Error	44	158937.06	3612.20			
Total	59					

The above table reveals that the variation in the actinomycetes population of the soil between different pine plantations is not significant but it is significant between different sampling periods.

Table - 4. Analysis of variance for the fungal population (Number of Fungi/gm dry soil x 10^3) of the soil of two different pine plantations for May 77 - April 78.

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value		Remarks
					5 %	1 %	
Between plantations	1	91.80	91.80	9.98	4.84	9.65	Significant at both 5% and 1% level of probabilities
Between sampling periods	11	384.81	34.98	3.80	1.97	3.77	Significant at both 5% and 1% level of probabilities
Error	11	101.10	9.19				
Total	23						

The above table reveals that the variation in the fungal population of the soil between two different pine plantations and between different sampling periods is statistically significant.

Table - 5. Analysis of variance for the bacterial population (Number of bacteria/gm dry soil x 10^5) of soil of two different pine plantations for May 77 - April 78.

Sources of variations	D.F	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value		Remarks
					5%	1%	
Between plantations	1	127.74	127.74	1.48	4.84	9.65	Not significant
Between sampling periods	11	10439.50	949.04	11.04	1.97	3.77	Significant at both 5% and 1% level of probabilities.
Error	11	945.57	85.96				
Total	23						

The above table reveals that the variation in the bacterial population of the soil between two different pine plantations is not significant but it is significant between different sampling periods.

Table - 6. Analysis of variance for the actinomycetes population (Number of actinomycetes/gm dry soil x 10^5) of soil of two different pine plantations for May 77 - April 78.

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value		Remarks
					5%	1%	
Between plantations	1	166.01	166.01	3.74	4.84	9.65	Not significant
Between sampling periods	11	9563.13	869.38	19.61	1.97	3.77	Significant at both 5% and 1% level of probabilities.
Error	11	487.65	44.33				
Total	23						

The above table reveals that the variation in the actinomycetes population of the soil between two different pine plantations is not significant but it is significant between different sampling periods.

Table - 7. Percentage relative abundance and frequency of fungal species recorded in soil of 1955 pineplantation, during 1976-77.

Sampling periods / Fungal species isolated	PERCENTAGE RELATIVE ABUNDANCE												Frequency
	APR76	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR 77	
Absidia cylindrospora	-	7.0	-	11.0	36.0	-	8.0	31.0	26.0	55.0	-	22.0	C
Circinella sp	-	3.0	-	-	-	3.0	-	-	-	-	-	-	R
Mucor hiemalis	-	-	-	-	-	-	2.0	-	-	-	3.0	-	R
Thannidium elegans	-	-	-	-	-	-	1.0	-	-	-	-	-	R
Cunninghamella echinulata	24.0	-	-	-	23.0	-	1.0	-	-	-	-	-	O
Pythium sp	-	3.0	100.0	76.0	-	78.0	82.0	40.0	69.0	31.0	-	25.0	C
Yellow unidentified ascomycetes?	-	-	-	-	-	-	-	-	2.0	-	-	-	R
Phoma humicola	-	-	-	-	-	9.0	-	6.0	-	-	-	-	R
Trichoderma viride	76.0	2.0	-	10.0	17.0	10.0	7.0	13.0	3.0	5.0	17.0	30.0	D
Aspergillus niger	-	1.0	-	-	-	-	-	-	-	-	1.0	-	R
Penicillium chrysogenum	-	-	-	2.0	-	-	-	10.0	-	-	70.0	23.0	O
Glocladium roseus	-	-	-	-	-	-	-	-	-	-	7.0	-	R
Verticillium sp	-	82.0	-	-	-	-	-	-	-	-	-	-	R
Cladosporium herbarum	-	1.0	-	-	-	-	-	-	-	-	-	-	R
Fusarium sporotrichoides	-	1.0	-	2.0	-	-	-	-	-	9.0	-	-	O
Sterile white mycelia	-	-	-	-	24.0	-	-	-	-	-	2.0	-	R

- = Absent. D = Dominant (81-100% frequency) O = Occasional (21-40% frequency)
C = Common (61-80% frequency) R = Rare (1-20% frequency)
F = Frequent (41-60% frequency).

Table - 8. Percentage relative abundance and frequency of fungal species recorded in soil of 1965 pine plantation, during 1976-77.

Fungal species isolated / Sampling periods	PERCENTAGE RELATIVE ABUNDANCE												Frequency
	APR76	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR 77	
Absidia cylindrospora	-	30.0	4.0	2.0	43.0	-	-	7.0	13.0	-	-	7.0	F
Mucor hiemalis	-	-	-	-	-	7.0	-	1.0	-	-	-	-	R
Zygorhynchus sp	-	-	-	-	-	-	-	-	-	2.0	-	-	R
Cunnighamella echinulata	56.0	-	-	-	18.0	-	6.0	-	-	-	-	-	O
Pythium sp	9.0	1.0	44.0	77.0	7.0	74.0	81.0	30.0	62.0	-	4.0	27.0	D
Chaetomium globosum	-	-	-	-	-	2.0	-	-	-	-	-	-	R
Phoma humicola	-	22.0	-	17.0	-	-	4.0	-	-	-	-	-	O
Trichoderma viride	19.0	6.0	3.0	2.0	19.0	11.0	8.0	17.0	4.0	2.0	25.0	44.0	D
Penicillium chrysogenum	16.0	6.0	49.0	2.0	-	3.0	1.0	34.0	21.0	25.0	70.0	22.0	D
Scopulariopsis sp	-	-	-	-	-	-	-	4.0	-	-	-	-	R
Gliocladium roseum	-	-	-	-	-	-	-	-	-	27.0	-	-	R
Verticillium sp	-	22.0	-	-	-	-	-	-	-	-	-	-	R
Alternaria tenuis	-	-	-	-	-	-	-	-	-	-	1.0	-	R
Fusarium sporotrichoides	-	13.0	-	-	-	3.0	-	-	-	44.0	-	-	O
Sterile white mycelia	-	-	-	-	13.0	-	-	7.0	-	-	-	-	R

Table - 9. Percentage relative abundance and frequency of fungal species recorded in soil of 1970 pine plantation during 1976-77.

Fungal species isolated / Sampling periods	PERCENTAGE RELATIVE ABUNDANCE												Frequency
	APR76	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR 77	
Absidia cylindrospora	-	16.0	20.0	55.0	56.0	19.0	15.0	29.0	25.0	49.0	-	56.0	D
Circinella sp	7.0	-	-	-	-	-	-	-	-	-	-	-	R
Mucor hiemalis	4.0	-	-	-	-	-	-	-	-	-	-	-	R
Pythyum sp	-	-	73.0	16.0	-	26.0	69.0	32.0	48.0	24.0	5.0	6.0	C
Chaetomium globosum	-	-	-	-	-	14.0	-	-	-	-	-	-	R
Phoma humicola	8.0	10.0	-	-	-	22.0	6.0	7.0	-	-	-	-	F
Trichoderma viride	8.0	7.0	-	27.0	29.0	15.0	10.0	25.0	-	-	3.0	13.0	C
Aspergillus niger	-	-	-	-	-	-	-	-	-	-	1.0	-	R
Penicillium chrysogenum	6.0	-	7.0	2.0	5.0	4.0	-	7.0	27.0	2.0	89.0	25.0	D
Verticillium sp	19.0	43.0	-	-	-	-	-	-	-	-	-	-	R
Cladosporium herbarum	-	-	-	-	-	-	-	-	-	-	2.0	-	R
Fusarium sporotrichoides	-	24.0	-	-	-	-	-	-	-	25.0	-	-	R
Sterile white mycelia	41.0	-	-	-	10.0	-	-	-	-	-	-	-	R
Unidentified black colonies?	7.0	-	-	-	-	-	-	-	-	-	-	-	R

Table - 10. Percentage relative abundance and frequency of fungal species recorded in soil of 1961 (High altitude) pine plantation during 1976-77.

Sampling periods / Fungal species isolated	PERCENTAGE RELATIVE ABUNDANCE												Frequency
	APR76	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR 77	
Absidia cylindrospora	30.0	25.0	4.0	8.0	51.0	24.0	15.0	17.0	23.0	3.0	-	31.0	D
Rhizopus nigricans	-	-	-	-	-	-	-	-	-	17.0	-	-	R
Cunninghamella echinulata	-	10.0	-	-	-	-	-	-	-	-	-	-	R
Pythium sp	39.0	1.0	94.0	76.0	-	60.0	74.0	38.0	64.0	38.0	-	40.0	D
Thielavia sp	-	8.0	-	-	-	-	-	-	-	-	-	-	R
Unidentified ascomycetes?	-	2.0	-	-	-	-	-	-	-	-	-	-	R
Phoma humicola	7.0	-	-	-	-	8.0	1.0	-	-	-	-	-	O
Trichoderma viride	23.0	12.0	-	16.0	12.0	7.0	10.0	43.0	4.0	-	6.0	19.0	D
Aspergillus niger	-	3.0	-	-	-	-	-	-	-	1.0	-	-	R
Penicillium chrysogenum	-	-	2.0	-	-	1.0	-	2.0	9.0	19.0	91.0	10.0	F
Verticillium sp	-	15.0	-	-	-	-	-	-	-	-	-	-	R
Alternaria tenuis	1.0	-	-	-	-	-	-	-	-	-	-	-	R
Fusarium sporotrichoides	-	24.0	-	-	-	-	-	-	-	22.0	-	-	R
Sterile white mycelia	-	-	-	-	37.0	-	-	-	-	-	3.0	-	R

Table - 11. Percentage relative abundance and frequency of fungal species recorded in soil of 1961 (Low altitude) pine plantation during 1976-77.

Sampling periods / Fungal species recorded	PERCENTAGE RELATIVE ABUNDANCE												Frequency
	APR 76	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR 77	
Absidia cylindrospora	-	-	-	14.0	62.0	41.0	7.0	15.0	17.0	48.0	-	17.0	C
Rhizopus nigricans	5.0	-	-	-	-	-	-	-	-	-	-	-	R
Circinella sp	-	-	-	-	-	3.0	-	-	-	-	-	-	R
Mucor hiemalis	-	-	-	-	-	-	-	1.0	-	-	4.0	4	R
Cunninghamella echinulata	-	-	-	-	6.0	-	-	-	-	-	-	-	R
Pythium sp	19.0	-	-	51.0	-	14.0	73.0	52.0	61.0	-	6.0	39.0	C
Unidentified phycomycetes ?	1.0	-	-	-	-	-	-	2.0	-	-	-	-	R
Phoma humicola	9.0	-	-	12.0	-	5.0	2.0	-	5.0	-	-	-	F
Trichoderma viride	20.0	-	-	18.0	12.0	37.0	18.0	28.0	10.0	-	26.0	37.0	D
Aspergillus niger	2.0	-	-	-	-	-	-	-	1.0	-	-	-	R
Penicillium chrysogenum	24.0	-	-	4.0	-	-	-	2.0	6.0	-	67.0	6.0	F
Verticillium sp	-	-	-	1.0	-	-	-	-	-	-	-	1.0	R
Cladosporium herbarum	20.0	-	-	-	-	-	-	-	-	-	-	-	R
Fusarium sporotrichoides	-	-	-	-	-	-	-	-	-	52.0	-	-	R
Sterile white mycelia	-	-	-	-	20.0	-	-	-	-	-	-	-	R

Table - 12. Analysis of variance for percentage relative abundance of different soil Fungal species of 1955 pine plantation during 1976-77.

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value		Remarks
					5%	1%	
Between fungal species	15	21529.3	1435.28	7.02	1.67	2.04	Significant
Error	176	35942.7	204.21				
Total	191						

The above table reveals that the variation in the percentage relative abundance of fungi between different speices is statistically significant.

Table - 13. Analysis of variance for percentage relative abundance of different Fungal species of 1965 pine plantation during 1976-77.

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value 5%	1%	Remarks
Between Fungal species	14	15787.0	1127.64	6.93	1.67	2.04	Significant
Error	165	26815.0	162.5				
Total	179						

The above table reveals that the variation in the percentage relative abundance of fungi between different species is statistically significant.

Table - 14. Analysis of variance for percentage relative abundance of different Fungal species of 1970 pine plantation during 1976-77.

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value		Remarks
					5%	1%	
Between fungal species	13	12596.58	1045.8	8.70	1.67	2.04	Significant
Error	154	18525.0	120.2				
Total	167						

The above table reveals that the variation in the percentage relative abundance of fungi between different species is statistically significant.

Table - 15. Analysis of variance for percentage relative abundance of different fungal species of 1961 plantation on high altitude during 1976-77.

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value		Remarks
					5%	1%	
Between fungal species	13	22567.58	1735.96	10.68	1.67	2.04	Significant
Error	154	25011.0	162.40				
Total	167						

The above table reveals that the variation in the percentage relative abundance of fungi between different species is statistically significant.

Table - 16. Analysis of variance for percentage relative abundance of different fungal species of 1961 plantation on low altitude, during 1976-1977.

Sources of variation	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value		Remarks
					5%	1%	
Between fungal species	14	11700.45	835.74	6.61	1.67	2.04	Significant
Error	165	22604.0	126.27				
Total	179						

The above table reveals that the variation in the percentage relative abundance of fungi between different species is statistically significant.

Table - 17. Percentage relative abundance and frequency of fungal species recorded in soil of 1955 pine plantation during 1977-78.

Sampling periods / Fungal species isolated	PERCENTAGE RELATIVE ABUNDANCE												FREQUENCY
	MAY 77	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR	APR 78	
Absidia cylindrospora	7.0	12.0	2.0	6.0	-	-	24.0	8.0	15.0	20.0	25.0	30.0	D
Circinella sp	19.0	11.0	-	1.0	-	-	-	8.0	15.0	15.0	20.0	25.0	C
Cunninghamella echinulata	-	-	-	-	-	5.0	-	-	-	-	-	-	R
Pythium sp	49.0	68.0	71.0	83.0	16.0	60.0	21.0	-	-	-	-	-	F
Gelasinospora sp	-	-	-	-	31.0	-	-	-	-	-	-	-	R
Sordaria sp	-	1.0	-	-	-	-	-	-	-	-	-	-	R
Phoma humicola	-	-	-	-	32.0	10.0	8.0	14.0	30.0	15.0	15.0	15.0	C
Trichoderma viride	25.0	8.0	27.0	10.0	21.0	25.0	47.0	70.0	40.0	50.0	40.0	30.0	D

Table - 18. Percentage relative abundance and frequency of fungal species recorded in soil of 1970 pine plantation during 1977-78.

Sampling periods / Fungal species isolated	PERCENTAGE RELATIVE ABUNDANCE												Frequency
	MAY 77	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR	APR78	
Absidia cylindrospora	33.0	2.0	8.0	32.0	-	-	24.0	11.0	44.0	50.0	30.0	18.0	D
Mucor hiemalis	-	-	-	2.0	-	2.0	-	3.0	-	-	-	-	O
Actinomucor sp	-	-	-	-	-	-	-	2.0	-	-	-	-	R
Cunninghamella echinulata	-	-	-	-	5.0	-	-	-	-	-	-	-	R
Pythium sp	4.0	37.0	26.0	44.0	19.0	64.0	21.0	3.0	3.0	10.0	20.0	5.0	D
Thielavia sp	-	3.0	-	-	-	-	-	-	-	-	-	-	R
Chaetomium globosum	24.0	-	43.0	-	-	-	-	-	-	-	-	9.0	O
Gelasinospora sp	-	-	-	-	4.0	-	-	-	-	-	-	-	R
Phoma humicola	18.0	-	2.0	3.0	65.0	7.0	8.0	37.0	17.0	5.0	10.0	36.0	D
Trichoderma viride	20.0	42.0	17.0	15.0	7.0	27.0	47.0	18.0	31.0	20.0	15.0	23.0	D
Penicillium chrysogenum	1.0	14.0	2.0	4.0	-	-	-	26.0	5.0	15.0	25.0	4.0	C
Gliocladium roseum	-	-	2.0	-	-	-	-	-	-	-	-	-	R
Sterile white mycelia	-	2.0	-	-	-	-	-	-	-	-	-	5.0	R

Table - 19. Analysis of variance for percentage relative abundance of different soil fungal species of 1955 pine plantation during 1977-78.

Sources of variation	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value		Remarks
					5%	1%	
Between fungal species	7	13781.5	1968.79	8.77	2.08	2.77	Significant at both 5% and 1% level of probabilities
Error	88	19754.5	224.48				
Total	95						

The above table reveals that the variation in the percentage relative abundance of fungi between different species is statistically significant.

Table - 20. Analysis of variance for percentage relative abundance of different soil fungal species of 1970 pine plantation during 1977-78.

Sources of variation	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value		Remarks
					5%	1%	
Between fungal species	12	13013.15	1084.42	9.70	1.75	2.18	Significant at both 5% and 1% level of probabilities
Error	143	15984.09	111.77				
Total	155						

The above table reveals that the variation in the percentage relative abundance of fungi between different species is statistically significant.

Table - 21. Distribution of soil fungi in summer season (April 76 - June 76) on the basis of percentage frequency.

Fungal species isolated	Pine plantations				
	1955	*1961(H.A.)	*1961(L.A.)	1965	1970
Absidia cylindrospora	33.3	100.0	-	66.6	66.6
Rhizopus nigricans	-	-	33.3	-	-
Circinella sp	33.3	-	-	-	33.3
Mucor hiemalis	-	-	-	-	33.3
Cunninghamella echinulata	33.3	33.3	-	33.3	-
Pythium sp	66.6	100.0	33.3	100.0	33.3
Thielavia sp	-	33.3	-	-	-
Yellow colonies ascomycetes form?	-	33.3	-	-	-
Phoma humicola	-	33.3	33.3	33.3	66.6
Trichoderma viride	66.6	66.6	33.3	100.0	66.6
Aspergillus niger	33.3	33.3	33.3	-	-
Penicillium chrysogenum	-	33.3	33.3	100.0	66.6
Verticillium sp	33.3	33.3	-	33.3	66.6
Cladosporium herbarum	33.3	33.3	33.3	-	-
Alternaria tenuis	-	33.3	-	-	-
Fusarium sprotrichoides	33.3	33.3	-	33.3	33.3
Sterile white mycelia	-	-	-	-	33.3

* H.A. = High altitude; L.A. = Low altitude.

Table - 22. Distribution of soil fungi in rainy season (July 76- Oct.76) on the basis of percentage frequency.

Fungal species isolated	Pine plantations				
	1955	*1961(H.A.)	*1961(L.A.)	1965	1970
Absidia cylindrospora	75.0	100.0	100.0	50.0	100.0
Circinella sp	25.0	-	25.0	-	-
Mucor hiemalis	25.0	-	-	25.0	-
Thamnidium elegans	25.0	-	-	-	-
Cunninghamella echinulata	50.0	-	25.0	50.0	-
Pythium sp	75.0	75.0	75.0	100.0	75.0
Chaetomium globosum	-	-	-	25.0	25.0
Phoma humicola	25.0	50.0	75.0	50.0	50.0
Trichoderma viride	100.0	100.0	100.0	100.0	100.0
Aspergillus niger	-	-	-	-	-
Penicillium chrysogenum	25.0	25.0	25.0	75.0	75.0
Verticillium sp	-	-	25.0	-	-
Fusarium sprotrichoides	25.0	-	-	25.0	-
Sterile white mycelia	25.0	25.0	25.0	25.0	25.0

Table - 23. Distribution of soil fungi in winter season (Nov.76-Feb.77) on the basis of percentage frequency.

Fungal species isolated	Pine plantations				
	1955	*1961 (H.A.)	*1961(L.A.)	1965	1970
Absidia cylindrospora	75.0	75.0	75.0	50.0	75.0
Rhizopus nigricans	-	25.0	-	-	-
Mucor hiemalis	25.0	-	50.0	25.0	-
Zygorhynchus sp	-	-	-	25.0	-
Pythium sp	75.0	75.0	75.0	75.0	100.0
Phoma humicola	25.0	-	25.0	-	25.0
Trichoderma viride	100.0	75.0	75.0	100.0	50.0
Aspergillus niger	25.0	25.0	25.0	-	25.0
Penicillium chrysogenum	50.0	100.0	75.0	100.0	100.0
Scopulariopsis sp	-	-	-	25.0	-
Gliocladium roseum	-	-	-	25.0	-
Cladosporium herbarum	-	-	-	-	25.0
Alternaria tenuis	-	-	-	25.0	-
Fusarium sporotrichoides	25.0	25.0	25.0	25.0	25.0
Sterile white mycelia	25.0	25.0	-	25.0	-

Table - 24. Distribution of soil fungi under two different pine plantations in summer, winter and rainy season on the basis of percentage frequency during 1977-78.

Fungal species isolated	1955 pine plantation			1970 pine plantation		
	SUMMER	WINTER	RAINY	SUMMER	WINTER	RAINY
Absidia cylindrospora	100.0	50.0	100.0	100.0	50.0	100.0
Circinella sp	100.0	25.0	75.0	-	-	-
Mucor hiemalis	-	-	-	-	50.0	25.0
Actinomucor sp	-	-	-	-	-	25.0
Cunninghamella echinulata	-	25.0	-	-	25.0	-
Pythium sp	100.0	100.0	25.0	100.0	100.0	100.0
Thielavia sp	-	-	-	50.0	-	-
Chaetomium globosum	-	-	-	50.0	25.0	-
Gelasinospora sp	-	25.0	-	-	25.0	-
Sordaria sp	50.0	-	-	-	-	-
Phoma humicola	-	50.0	100.0	50.0	100.0	100.0
Trichoderma viride	100.0	100.0	100.0	100.0	100.0	100.0
Penicillium chrysogenum	-	-	-	100.0	50.0	75.0
Gliocladium roseum	-	-	-	-	25.0	-
Sterile white mycelia	-	-	-	50.0	-	-

Table - 25. Moisture content of soil collected at different sampling periods under different pine plantations, during 1976-1977.

Sampling periods / plantations	Apr 76	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar 77	Total
1955	20.0	30.0	25.0	24.0	28.0	28.0	25.0	22.0	22.5	19.0	13.0	20.0	276.5
1965	15.0	26.0	25.0	20.0	30.0	28.0	25.0	21.0	23.5	20.0	16.0	23.0	272.5
1970	21.0	21.0	25.0	20.0	27.0	25.0	23.0	15.0	21.0	15.0	11.0	23.0	247.0
*1961 (H.A.)	20.0	27.0	27.0	26.0	32.0	30.0	26.0	18.0	22.5	18.0	11.0	25.0	282.5
*1961 (L.A.)	20.0	27.0	29.0	20.0	30.0	27.0	25.0	20.0	25.0	17.0	16.0	23.0	279.0
Total	96.0	131.0	131.0	110.0	147.0	138.0	124.0	96.0	114.0	89.0	67.0	114.0	1357.5

H.A. = High altitude

L.A. = Low altitude

Table - 26. Analysis of variance for moisture content of soil collected at different sampling periods under different pine plantations for 1976-1977.

Sources of variation	D.F.	S.S.	M.S,S.	Variance ratio calculated	Table 'F' value 5%	Remarks
Between plantations	4	66.96	16.74	4.370	2.592	Significant
Between sampling periods	11	1166.42	106.03	27.684	2.029	Significant
Error	44	168.94	3.83			
Total	59					

– The above table reveals that the variation in the moisture content of the soil between different pine plantations and between different sampling periods is statistically significant.

Table - 27. Moisture content of soil collected at different sampling periods under two pine plantations during 1977-1978.

Sampling periods / Plantations	MAY 77	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR	APR 78	TOTAL
1955	32.8	28.6	40.0	31.2	30.0	29.4	30.0	23.7	20.0	20,5	20.5	21.0	337.2
1970	35.0	31.4	38.0	32.5	34.0	36.0	23.0	23.0	23.0	20.0	20.0	20.5	352.8
Total	67.8	60.0	78.0	63.7	64.0	65.8	66.0	53.0	46.7	43.0	40.5	41.5	690.0

Table - 28. Analysis of variance for moisture content of soil collected at different sampling periods under two pine plantations for 1977-1978.

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value		Remarks
					5%	1%	
Between plantations	1	10.14	10.14	1.41	4.84	9.65	Not significant
Between sampling periods	11	823.28	74.84	10.41	1.97	3.77	Significant at both 5% and 1% level of probabilities
Error	11	79.04	7.19				
Total	23						

The above table reveals that the variation in the moisture content of the soil between two different pine plantations is not significant but it is statistically significant between different sampling periods.

Table - 29 . pH of the soil collected at different sampling periods under different pine plantations during 1976-1977.

Plantations \ Sampling periods	APR 1976	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN 1977	FEB	MAR	Total
1955	5.85	5.95	5.80	6.25	5.95	6.15	5.83	6.35	6.50	6.20	5.80	6.30	72.93
1965	6.20	6.05	5.70	5.70	5.62	5.65	5.75	6.00	4.60	6.60	6.30	6.40	70.57
1970	6.25	6.25	5.70	4.70	5.55	5.95	6.35	6.65	5.10	6.50	6.40	6.80	72.20
* 1961 (H.A.)	6.00	6.15	5.65	6.40	5.86	6.00	6.45	6.65	6.10	6.60	5.55	6.75	74.26
* 1961 (L.A.)	5.90	5.95	5.90	5.80	5.92	5.85	6.15	6.85	6.20	6.30	6.00	6.70	73.52
Total	30.50	30.35	28.75	28.85	28.90	29.60	30.53	32.50	28.50	32.20	30.05	32.95	

H.A. = High altitude

L.A. = Low altitude.

Table - 30. Analysis of variance for pH of the soil collected at different sampling periods under different pine plantations for 1976-1977.

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value 5%	Remarks
Between plantations	4	0.66	0.165	0.597	2.592	Not significant
Between sampling periods	11	5.16	0.469	1.699	2.029	Not significant
Error	44	12.16	0.276			
Total	59					

The above table reveals that the variation in the pH of the soil between different pine plantations and between different sampling periods is statistically not significant.

Table - 31. pH of the soil collected at different sampling periods under two pine plantations during 1977-1978.

Sampling periods	MAY 77	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR	APR 77	TOTAL
1955	5.85	5.70	6.45	5.87	6.32	5.03	6.35	6.00	6.54	6.00	6.25	6.00	72.36
1970	5.95	6.25	5.40	4.31	5.51	5.87	6.60	5.35	6.12	6.50	6.87	5.95	70.68
Total	11.80	11.95	11.85	10.18	11.83	10.90	12.95	11.35	12.66	12.50	13.12	11.95	143.04

Table - 32. Analysis of variance for pH of the soil collected at different sampling periods under two pine plantations for 1977-78.

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value		Remarks
					5%	1%	
Between plantations	1	0.12	0.12	0.75	4.84	9.65	Not significant
Between sampling periods	11	3.90	0.35	2.19	1.97	3.77	Significant at 5% level of probabilities.
Error	11	1.77	0.16				
Total	23						

The above table reveals that the variation in the pH of the soil between two different plantations is not significant but is significant between different sampling periods.

Table - 33. Correlation coefficients (r) for total number of microbes, dehydrogenase activity with and without glucose, Co_2 evolution, pH, moisture content, organic matter and nitrogen of soil of 1970 pine plantation observed during 1977-1978.

Sources of variations	D.F.	Co_2 evolution	Dehydrogenase activity with glucose	Dehydrogenase activity without glucose	Total number of microbes
Total number of microbes	10	0.732**	- 0.181	- 0.121	
Dehydrogenase activity with glucose	10	- 0.156	-	0.949***	
Soil pH	10	- 0.292	- 0.696*	-	- 0.526
Soil moisture	10	0.362	- 0.764**	-	0.079
Soil organic matter	10	0.090	0.192	-	0.242
Soil nitrogen	10	- 0.174	0.027	-	0.028

*, **, *** = $P < 0.05$, 0.01, 0.001 respectively.

All properties were calculated on the basis of dry weight soil.

Table - 34. Percentage of nitrogen present in soil of 1970 pine plantation during 1977-1978.

Sampling periods	% of Nitrogen in soil
May 1977	0.237
June "	0.238
July "	0.228
August "	0.231
September"	0.219
October "	0.204
November "	0.221
December "	0.236
January 1978	0.224
February "	0.224
March "	0.231
April "	0.255

DISCUSSION

A perusal of the tables 1-3 and 5-6, suggests that statistically no significant variation was observed in the soil microbial population (Fungi, bacteria and actinomycetes) between different age groups of pine plantations due to the insignificant variation in the pH of the soil between different plantations (Tables 30,32). This may be also due to almost a similar type of the soil irrespective of different age groups of Pine stands. Further a thick uniform slowly decomposing layer of surface litter remains throughout the year in all the plantations which conceivably buffers the underlying mineral horizons against rapid change and acts as mulching and the source of nutrients to the microbes. The condition which might have checked the population variation between different plantations. Jones and Richards, (1977) in their studies of the changes in the soil microflora of dry sclerophyll forest sites in South-East Queensland following reafforestation with exotic pines also showed no significant changes in bacterial or actinomycetes population densities between different age groups of pine plantations. They found however, that the numbers of fungi increased rapidly to $4.3 \times 10^5 g^{-1}$,15 years after planting, and remained at this level thereafter.

The significant seasonal changes was observed in the fungal, bacterial and actinomycetes population of the soil of this region (Figs.1-5 and Tables 1-6). Extreme climatic fluctuations observed during the two years course of investigation might have exerted variation

in the soil microbial population at different sampling (Fig.13). Wright and Bollen (1961) while working on microflora of Douglass fir forest soil, observed that there was extensive monthly fluctuation in number of microorganisms and the latter greatly increased during the rainy periods and decreased during drought. A low count of fungi, bacteria and actinomycetes was observed during November to January (Figs.1-5). During this period the maximum and minimum temperature of atmosphere ranged between 18°c - 15°c and 11°c - 5°c respectively with very scanty rainfall (Fig.13). Such conditions perhaps did not prove conducive for the growth of microbes in soil. Comparatively high counts of fungi, bacteria and actinomycetes was observed during the months of May, August and September when rainfall and the temperature were conducive for the growth of soil microbes (Figs.1-5). Low counts in the soil microbial population again during the months of June and July, may be explained in the light of high rainfall during these periods, which might have leached the available nutrients (soluble forms) from the upper horizon of the soil (from which the counts were done) to lower horizon and thus affecting the soil microbes in the upper horizon (Figs.1-5). Such a low microflora was also observed in case of two forest localities of this region by Mishra et al (1977) and they suggested that this may be due to washing away of the plant material from the slppes along with rains during the periods. Waksman (1952) indicated that the distribution of microorganisms in the soil is controlled by numerous ecological factors, comprising climatic or atmospheric, edaphic or soil, and biotic or living.

Earlier workers have shown that fungi flourish in acid soils and bacteria in alkaline. Later workers, viz; Jensen (1931), Warcup (1951), Saksena (1955), Dwivedi (1959) and Mishra (1964) have reported the occurence of fungi in good number both in acidic and alkaline soils. Williams et al (1971) suggested that some group of actinomycetes are more wide spread in acid soils. Warcup (1951) distinguished two main groups of fungi - those occuring in acidic soils and those preferring alkaline soils. Most fungi have the optimum growth at neutral reaction or slightly on the acidic side (Hall, 1933; Howker,1950). An initial pH of 5 - 6 is satisfactory (not necessarily optimum) for the majority of the fungi (Lily and Barnett,1951). Apparently it was suggested that pH of the soils was not a factor contributing to mycofloral changes (Menon and Williams,1957). It is evident from the above description as well as from the result depicted in the tables that slight variations in soil pH, did not exert any remarkable influence on the microbial population (Tables 29,32). Goodfellow et al (1968) also could not establish any correlation between bacterial population, pH, temperature and percentage moisture content of a pine forest soil by dilution plate counts.

Saksena (1955), Dwivedi (1959), Mishra (1964) and Mishra et al (1972) found a direct correlation between moisture content of soil and fungal population while Ghosh and Dutta (1960) could not establish such correlation.

However, from the present study no correlation was found between fungi, bacteria, actinomycetes and moisture content of pine forest of this region. The methods employed

for counting are probably not sufficiently sensitive to detect correlations. Jones and Richards (1977) concluded that changes in the soil mycoflora of Pine are caused partly by some other factors associated with pines, and not simply as a result of their influence on litter. The rate of accumulation and breakdown on the plantation floor are also likely to influence the microbial population. He also observed a large seasonal fluctuation in the population of major groups of soil microbes in different pine plantations.

Not much variation was observed in the quality of soil fungi of different plantations (Tables 7-11). Similar nature of the soil of all the plantations may be responsible for such similarity. The spread of slowly decomposing surface litter on the forest floor of all the plantations also contribute for this similarity. Widespread occurence of *Absidia cylindrospora*, *Pythium* sp. and *Cunninghamella echinulata* during summer and rainy seasons suggests that they remain passive during other season and on the onset of summer and rain they become active soil saprophytes and colonize more profusely the dead organic matter on soil (Tables 21-24). However, their higher incidence in soil during summer and rainy season can be explained on the basis of increase in the soil moisture content due to higher rainfall from the month of May to August (Tables 25 and 29). During these months more soluble nutrients were perhaps available due to increased decomposition of pine litter. Mc-Laughlin (1947), too, has shown that the frequency of isolation of *Pythium* from soil is strongly correlated with high soil moisture content.

Barton (1960) has shown a direct correlation between the saprophytic colonization of substrate in soil and moisture content by _Pythium mammilatum_.

Earlier workers, viz; Waksman (1917), Paine (1927), Galloway (1936) and Dwivedi (1959) suggested that mucorales and other genera of phycomycetes are generally less common in grassland soils but are abundant in forest soils. Christensen _et al_ (1962) also isolated several species of phycomycetes particularly _Absidia_ sp. from the soil of Wet-mesic forests in Southern Wisconsin. Widden and Parkinson (1973) also isolated regularly _Absidia_, _Mucor_, _Zygorhynchus_ and several other species of phycomycetes from the soil of _Pinus resinosa_, _Pinus banksiana_, _Pinus strobus_ and _Pinus contorta_ plantations. Mishra and Kanaujia (1973) isolated regularly _Absidia_ and _Mucor_ from the rhizosphere and nonrhizosphere soil of seven gymnosperms, of which _Pinus longifolia_ was one. Vaartaja (1975) reported a widespread occurence of _Pythium sylvaticum_ in Canadian forest nurseries and the fungus was more common in forest soils of _Pinus resinosa_.

Though the class ascomycetes and basidiomycetes play an important role in the decomposition of organic matter especially in forest soils and although several hundred spp. of the group are present in soils, their isolation is so difficult that our knowledge of the hyphal growth and distribution in different kinds of habitats is sparse, During the present investigation the class ascomycetes was very poorly represented from the pine forest soil which suggests that either the technique

employed was not sufficiently sensitive for their isolation or they may not have succeeded in finding a place in the plates due to presence of the fast growing forms like *Trichoderma viride* and *Penicillium* sp., *Absidia cylindrospora* and *Pythium* sp.

The majority of the colonies and species isolated belonged to the class deuteromycetes (Tables 7-11 and 17-18). Members of this group are pronounced cellulose decomposer which multiply and grow in soil with organic matter. In the forest soil with enough organic matter the presence of this class of fungi is well expected.

Trichoderma viride and *Penicillium chrysogenum* were repeatedly isolated from the soil of almost all the pine plantations and they were present in the soil throughout the year (Figs.6-12). This suggests that these species are the active cellulose decomposer of this forest soil whose growth is being supported by the presence of uniform surface litter throughout the season.

The genus *Aspergillus* was represented very poorly and also could not be isolated from all the plantations. This is in accordance with the earlier views that Aspergilli are abundant in warmer regions of the world and *Penicillia* in colder regions (Jensen,1931 and Garrett, 1956). Since the atmospheric temperature of this region remains very cold from November to January the above proposition is well documented (Fig.13). *Trichoderma viride* was reported by Warcup (1951) to be common in acidic soils which is in agreement with the present study. Mishra (1964) also isolated this genus repeatedly from

waterlogged acidic soils. The forest soil being acidic, the growth of Trichoderma viride in the area is logical. Jones and Richards (1977) also noted that Penicillium and Trichoderma sp. were much more common in the soil of pine plantations than in native forest soil of southeast Queensland.

Fusarium sporotrichoides though isolated during present investigation, was practically noted as rare species. The acidic nature of the pine forest soil probably did not favour the growth of this species. Several workers have also reported that Fusarium does not exist or survive in coniferous soils (Park, 1954; Smith, 1967; Toussoun et al 1969). Freddi et al (1975) confirmed that presence of some toxic acids in the pine forest soil is partly responsible for the absence of Fusarium sp. from these soils. During the present investigation several types of phytotoxic compounds were detected from the decomposing litter of Pinus kesiya which probably inhibited the growth of such species.

From the biochemical studies, statistically no correlation between dehydrogenase activities and total microbial population of the soil was observed (Fig.14, Table 33). This suggests that the two parameters are governed by different environmental factors. Stevenson (1959) also could not correlate dehydrogenase activity and bacterial numbers. However, he noted that two parameters were parrallel during decomposition of flax residues added to one of the soils. Hirte (1963) and Gilot et al (1967) also did not find any such correlation

in other soils.

In the past few years a number of investigators have devoted considerable time to the development of enzymatic tests for determining microbial activity in soil. However, in most instances no relationship could be found between the enzyme tests and total number of microorganisms (Drobnik et al 1955; Koepf, 1954). Ross (1973) also studied dehydrogenase activities in hard beech forest soil and could not establish any significant correlation with numbers of viable bacteria.

A substantial increase in the dehydrogenase activities was observed in the soil by addition of glucose as a substrate (Fig.14). This indicates that the presence of carbohydrates (glucose) in the soil can markedly influence the microbial metabolism in natural condition by increasing the efficiency of the enzyme system. This result is in agreement with the findings of Galstyan (1964); Kiss et al (1965); Thalman (1968); and Ross (1970).

Statistically a positive correlation between Co_2 evolution and total microbial population of the soil was observed (Table 33). This suggests that the quantity of Co_2 evolution from the soil is partly dependent on the quantity of microbes present per unit area and also on the quantity of substrate degraded by the action of microbes in the soil. Earlier works of Witkamp (1966) and Bauzon et al (1969) also indicated a positive correlations of Co_2 production with numbers of bacteria in certain forest soils. Jung (1970), on the other hand, found significant correlations of Co_2 production with fungi in other forest soil.

From the present investigation no correlation could be established between dehydrogenase activities and Co_2 evolution from the soil (Table 33). Both the activities differ significantly under varying environmental situations at different time periods. This could not be accounted due to the limitations of the methods under natural condition. Schaefer (1963); Gilot et al (1967); Bauzen et al (1969) and Ross et al (1970) did not find any correlation between dehydrogenase activity and Co_2 production in soils.

A significant correlation between dehydrogenase activities, soil moisture and pH suggests that enzyme activities in soil is markedly influenced by certain optimal range of pH and moisture content of a particular field soil (Table 33). Moreover under natural conditions, levels of dehydrogenase activities of soil may be limited by these two factors. Ross et al (1970), also observed a significant correlation between dehydrogenase activities, soil moisture, and pH of soil at New Zealand under pasture.

Though the effect of moisture content on Co_2 evolution was apparent from Fig.15, yet no significant correlation was observed between the parameters (Table 33). This indicates that either evolution of Co_2 from the soil is not solely governed by moisture content or method of Co_2 determination under field condition, is not sufficiently sensitive to detect any correlation. Edwards (1975) also could not observe any significant correlation between moisture and Co_2 evolution rates of a mixed deciduous forest soil.

Stevenson (1959) observed that microbial activity

of soil fluctuates due to the decomposition of the organic material and corresponding changes occur in dehydrogenase activity and soil respiration (Oxygen uptake and Co_2 evolution). In the present study no correlation, however, could be observed between organic matter content, Co_2 evolution, dehydrogenase activity and microbial population (Table 33). The possible explanation for such situation may be the presence of uniform quantity of organic matter in pine forest soil throughout the observation and also due to the slow rate of decomposition of organic matter in this forest (Fig.15).

Soil nitrogen did not effect Co_2 evolution, dehydrogenase activities and total number of microbes at all (Table 33). The presence of soil nitrogen in this forest soil remained almost uniform throughout the year (Table 34). Minor fluctuation in the nitrogen content might not hage affected the soil microbial population, Co_2 evolution and dehydrogenase activities.

Chapter - 2

ECOLOGY OF LEAF SURFACE MICROORGANISMS OF PINE NEEDLES.

INTRODUCTION

The aerial surfaces of higher plants growing under natural conditions are usually associated with large and varied population of microorganisms. Some of these organisms are able to grow extensively on the surface of healthy plants while others are apparently only able to grow beyond the limitations imposed by their endogenous nutrients when the tissues on which they occur begin to senesce or are physically or physiologically damaged (Dickinson,1976). The term 'Phyllosphere' was introduced by Last (1955) to denote the leaf surface of plants, a term analogous to rhizosphere of Hiltner (1904). Last further advocated that it is also nutritionally rich microhabitat and provides a suitable substrate for the colonization and multiplication of microorganisms. Later on Dickinson (1965) used the term 'Phylloplane' for phyllosphere of Last.

Contrary to a generally held belief. healthy green leaves are superficially colonized by a colourful array of saprophytes many of which rarely occur in other ecological niches. The saprophytic surface flora is of interest in different respects such as its possible antagonistic action against plant parasites, its importance to degrade plant waxes, to produce plant growth regulators, to decompose plant material after leaf fall, to trigger plants to produce phytoalexins, its toxic properties for cattle, its role in nitrogen fixation and its function as a source for allergenic airborne spores. However, an understanding of their significance under field conditions and their possible role in leaf litter decomposition demands further research. The population of the world depends largely

upon the production of actively photosynthesizing leaves. Surprisingly, however, little was known of the immediate environment of leaves until interest was recently diverted by progress in our knowledge of three areas; the nature of the leaf surfaces, the energy budget of plants and the role played by the atmosphere as a medium in which a variety of substances and particles is suspended.

The existence of a three membered biological system host, parasite and saprophyte has only recently been accepted as responsible for events on leaf surface. After the development of efficient trapping devices as an aid to measure aerial dispersal of plant pathogens, it was soon realized that recognizable spores of plant pathogens formed only a small part of the total load of airborne particles, their number being augmented by spores and hyphal fragments of a diverse array of other microbes, pollen grains and atmospheric pollutants. Field assessments of the actimity of leaf surface microorganisms in vitro are difficult to make. It is relatively easy to show in vitro that some saprophytes can erode leaf waxes which increases the permeability. It is, however, much more difficult to ascertain under field conditions the role of the saprophytes in regulating the permeability of leaf. The same can be said of the activities of such saprophytes in influencing the activities of plant pathogens and their possible role in the decomposition of leaf litter in nature. Our present knowledge is not sufficient to trigger awareness. Future research may enable us to manipulate these

saprophytes in a predetermined manner.

Different methods have been used to assess populations of leaf surface saprophytes, but none alone has proved adequate. Generally, techniques involved in such studies are leaf washing and maceration which are ultimately cultured on agar media. The methods are useful and they facilitate the identification of colonies but they rarely enable true colonizers to be separated from chance contaminants.

The present work reports a systematic investigation of the mycoflora of Pine needles (*Pinus kesiya* Royle) of North-Eastern India and also the population dynamics of yeast. The findings include information regarding succession of the microbes on needles of different ages.

The purpose of the phylloplane studies can briefly be described as follows :-

1) To understand the presence of initial fungal colonizers on the leaf surface.
2) To understand the source of microbes responsible for litter decomposition.
3) To observe the pattern of fungal succession during litter decomposition.
4) To assess the role of phylloplane microorganisms in the litter decomposition.

REVIEW OF LITERATURE

Brown (1922) in his experiment on leaf surface elegantly demonstrated that microbes could benefit from nutrients available on the surfaces of leaves. A very great range of materials including inorganic ions, free sugars, amino acids and growth regulating gibberellins have been detected in leachates. Leaf surface saprophytes may affect the development of pathogens by the prior stimulation of the host to produce *phytoalexins. Such a material was originally defined by* Muller and Borger (1940) as an antibiotic which is produced as a result of the interaction of two metabolic systems, the host and the parasite and which inhibits the growth of microorganisms pathogenic to plants.

The interaction of leaf surface microorganisms, is of paramount importance to understand the ecology of leaf surface microorganisms. The balancing mechanisms operates, however, not only between the major group of microorganisms but also within them. Last (1955) demonstrated that the population of the pink yeast Sporobolomyces having previously been isolated from the entire surface of wheat leaves was suddenly found restricted to the margins of the wheat leaves.

Ruinen (1961) on the basis of his studies of the phylloplane flora of rainforest plants concluded that bacteria were the first colonizers of young leaves, and buds were the active sites of colonization. Ageing of the leaves results in a gradual increase of microbial cells. Ruinen (1961) also indicated that bacterial numbers

usually increased significantly before appreciable numbers of yeasts and filamentous fungi colonized green leaves. This view is generally accepted although it lacks confirmation.

The living leaf may be colonized by population of parasites and saprophytes which form the first stage of the succession on litter. Examinations of living leaves for the determination of the components of the early stages of mycofloral succession has been carried out by Kendrick and Burges (1962), Hering (1965) and Hogg and Hudson (1966).

Colonization of leaf surface by bacteria, yeasts and fungi has been a subject of research by several investigators. Last and Deighton (1965), Leben (1965), Sinha (1965) and Mangenot (1966) have reviewed such studies.

In the Southern hemisphere Macauley and Thrower (1966) studied the parasitic mycoflora within freshly fallen green leaves of *Eucalyptus regnans*. Hogg and Hudson (1966) examined young living leaves of *Fagus sylvatica* and found that the first colonizer, *Discula quercina* (conidial state of *Gnomonia errubunda*) appeared within 3 months of unfolding of the leaves. Dickinson (1967) studied the fungal colonization on the leaf surface of *Pisum sativum*. He concluded that newly formed leaves were not rapidly colonized by mycelial fungi. Hudson (1968) has emphasized the value of the studies of leaf surface fungi to investigate the mycoflora of living leaves on the tree.

Wardlaw (1968) observed that active bud is almost totally dependent on other parts of the plant for nutrients, which may be preferentially drawn into the bud. It is possible that some of these nutrients are available for the growth of microorganisms in spaces which remain wet in the bud. Davenport (1967,1968,1969) in England studied the important role of the apple bud in the seasonal cycle of epiphytic yeasts. The role of yeasts in the phylloplane is very important and actually very little work has been done on this aspect of leaf surface microbiology. Hislop and Cox (1969) examined expanding apple buds as part of their study of phylloplane fungi and yeasts.

In recent years our understanding of the complex surface configuration of leaves has increased (Martin and Juniper, 1970). The cuticular membrane, the external covering of epidermal cells now recognized to consist of several more or less distinct layers, is of paramount importance in determining permeability. The development of microbes is effected in a variety of ways by physical nature of the leaf surface. Firstly it will influence the depth of the boundary layer which affords protection to deposited propagules. It affects the balance of cuticular to stomatal transpiration and influences the reflectance of leaf and therefore its microclimate (Last and Warren, 1972). At present little is known about the way in which the superficial layers of wax are developed. Rentschler (1971) demonstrated that wax production can be stopped irreversibly at a relatively early stage of leaf development by particulate polutants and fungi. The chemical nature of the leaf surface invariably affects the

growth and development of surface propagules. Pugh and Buckley (1971) surveyed colonization of leaf surface by bacteria, yeasts and fungi. An excellent survey of research on phylloplane is represented by Preece and Dickinson (1971) in the proceedings of a symposium entitled 'Ecology of leaf surface microorganisms'.

Bailey (1971) suggested that the prior colonization of leaf surface by saprophytes may trigger the release of phytoalexins capable of decreasing the germination and subsequent attack by pathogens. Minerals, free sugars, amino acids, growth regulating substances and vitamins have been detected in leachings from plant leaves by Tukey (1971).

Fungi in New Zealand pastures have been studied by di Menna and Parle (1970) and McKenzie (1971). The effect of pollen on the leaf surface saprophytes has been worked out by Fokkema (1971). It was noted that effect of the pollen in the phyllosphere may be two fold : first the saprophytic microflora present on leaves will be levelled upto a higher density, secondly, the infection by some perthotropic pathogens, if present, may be enhanced. Stimulation will probably occur if the pathogens exhibit certain superficial development before penetration. The consequence of pollen on the development of plant diseases may depend on the presence of an already established saprophytic microflora on the plant surfaces, on which pollen lands.

Blakeman and Fraser (1971) have indicated that

microbes can add to the pool of available nutrients. They found that spores of *Botrytis cinerea* and *Mycosphaerella liquilicola* leak amino acids, and carbohydrates, a process occuring sooner with the former than with the latter.

Little is known about the interaction of phylloplane bacteria with other surface colonists. Phylloplane bacteria have been implicated with the antagonism of both plant pathogenic bacteria and fungi (Crosse, 1971; Fraser, 1971) but the classification of these bacteria has been unsatisfactory and consequently, it is difficult to determine the identity of the antagonists and hence estimate their distribution in the natural environment.

Mackenzie (1971) investigated the seasonal incidence, and possible interrelations of parasite and saprophytic fungi in Ryegrass and white clover pasture in New Zealand. In India Mishra and Srivastava (1971 a,b,c,d) investigated the leaf surface microflora of various crop plants but no such work on forest trees is available. Last and Warren (1972a) studied the form and functions of nonparasitic microbes colonizing green leaves. Bainbridge and Dickinson (1972) studied the effect of fungicides on the mycoflora of potato leaves from the time of shoot emergence to leaf senescence. Warren (1972a) studied the effect of pollen on leaf surface mycoflora of sugarbeet and observed that the main colonizers grouped into pink yeasts, white yeasts, *Cladosporium* spp and *Aureobasidium pullulans*, were all influenced by the natural presence of

pollen and reached high numbers on leaves in the plot bearing flowers. Warren (1972 b) provided circumstantial evidence to show that population of microorganisms enhanced by pollen could inhibit the development of *Phoma batae* on sugarbeet leaves.

Fokkema (1973) studied the antagonism of saprophytes against pathogens on agar plates and on Rye leaves with pollen. The phenomenon of interaction of *Alternaria porri* and saprophytic mycoflora of onion leaves was demonstrated by Fokkema *et al* (1976). Fokkema (1976) also highlightened many more phenomenon of antagonism of fungal saprophytes and pathogens an aerial plant surfaces. Warren (1976) studied the control of population of different yeasts and *Cladosporium* spp. on the leaves of maize and broadbean leaflets. During the course of investigations of leaf surface fungi on wheat (*Triticum aestivum*) Mishra and Tewari (1976) observed that certain saprophytes inhibited spore germination of *Puccinia graminis tritici*. This observation prompted them to extend their studies to explore the possibilities of biological control of the rust.

Collins and Hayes (1976) studied the seasonal incidence of microbes on the surface of first year needles of *Pinus abies* (Norway spruce) in Castle over forest, Dimfrieshire. The saprophytic microflora isolated from bud and needles during first year season of growth was characterized by a rapid increase in numbers to a peak value shortly after needle flushing. The pattern of succession and distribution of microfungi on attached

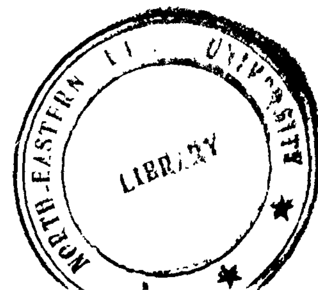
leaves of *Hippophae rhamnoides* was studied by Lindsey and Pugh (1976 a,b). They noted the complex nature of the leaf surface with its dense covering of trichomes and the nonrandom distribution of fungi. The development of yeast *Sporobolomyces roseus* on wheat leaf surface was studied under various microclimate of leaf surface (Bashi and Fokkema, 1977). They found that *Sporobolomyces roseus* developed on green wheat leaves grows equally well in stimulated dew as under high ambient relative humidity. Dickinson and O'Donell (1977) studied the behaviour of phylloplane fungi on *Phaseolus* leaves. Their results support the suggestion that humidity is a prime factor in determining the extent of leaf colonization. Latch *et al* (1977) studied the fungal flora of Rye grass swards in Wale and found that *Phoma* spp were the most commonly isolated fungi, followed by *Acremonium* spp, *Cladosporium* spp and *Tricellula aquatica*.

Brian *et al* (1977) studied the antagonistic interactions of phylloplane bacteria with *Drechslera dictyoides* in vitro and vivo experiments. It was deduced that their mode of activity included an initial inhibition of spore germination, a retardation in the rate of germ tube elongation and ultimately lysis of the hyphae. Forster (1977) studied the effect of leaf surface wax on the deposition of airborne propagules and concluded that deposition of wax has significant effect in increasing the capture of propagules from the atmosphere and in the establishment of the phylloplane flora.

Studies on leaf surface microflora colonizing the

pine needles at different stages of growth and their role in pine litter decomposition is lacking for our country. Therefore present investigation was undertaken.

MATERIALS AND METHODS


NORTH-EASTERN HILL UNIVERSITY
LIBRARY

The present investigation was carried out in the same experimental forest where the soil microflora was studied. The detailed description of the forest site is given in the chapter general introduction. In this investigation two parameters were considered :

(1) Dynamics of Fungal population of green and senescent pine needles - The experiment was conducted for a period of eleven months. Two pine plantations (1955 and 1970) were selected for the collection of the needles for isolation of mycoflora. The aim of this experiment was to assess the mycoflora of green and senescent needles, and the effect of ageing of the needles on the quality and quantity of leaf surface fungi. The experiment was conducted in case of two age groups of plantations. Senescent and green pine needles from both the plantations were collected from the month of November 76 upto September 77 at two months interval. Five trees in each case were selected for the sampling and the needles after random collection were mixed thoroughly in an aseptic condition.

(2) Fungal population of leaf surface of pine needles from folding to senescent to litter stages -

This experiment was performed for a period of two years till the needles entered into the litter stage. The experiment was performed in 1970 pine plantation only. The aim of this experiment was to evaluate the saprophytic activity on the leaf surface of Pine during different degrees of maturity of the

needles and to assess the pattern of fungal succession on needles before and after leaf fall. The overall aim of the experiment was to determine the nature of initial colonizers on leaf surface and their role in pine litter decomposition, under natural condition after leaf fall.

The experiment was conducted from the month of March 1976 to April 1978. Five pine trees of equal height were selected to collect the leaf samples throughout the study period. From each tree, samples were collected at random from approximately equal height of the trees.

Five replicate samples were collected when the needles were at different degrees of maturity after flushing as folding (bud), unfolding (buds opened) and young expanding green needles upto the needles which entered into the litter stage.

The first, second and third samplings were done on 25th.March, 25th.April and 25th.July 1976 respectively, when the needles were in folding (bud), unfolding (buds opened) and young expanding green stages. The fourth sampling of the needles was done on 25th.November 76 when the needles were mature. Subsequent samplings of the needles from senescent to litter stages were done at one month interval for a period of sixteen months upto April 1978 till the needles entered into the litter stage. The samples collected from the month of January 1977 upto September 1977 were found to be senescent. Collection of the needles as pine litter was

followed from the month of October 77 to April 78.

Collection of the samples - During each collection the samples were collected in previously sterilized polythene bags using sterilized scissors and forceps. The bags were properly sealed and transported to the laboratory and the leaves were used on the same day for the isolation of mycoflora. On a few occasions when isolation could not be done the sameday, the sample were stored overnight at 4°C (Ruscoe 1971). Additional samples were collected for the determination of moisture content and pH.

Isolation of Mycoflora - A combination of cultural isolation and direct microscopic methods was employed as advocated by Dickinson (1967,1971) and Last and Warren (1972) to get a comprehensive picture of the microflora. At first direct microscopy of the needles was done for several times but the fungi could not be identified due to lack of adequate mycelia and fruiting bodies. Only presence of spores was encountered during the direct microscopy and therefore the needles were cut into pieces and incubated on the surface of Rose bengal agar medium to enhance the growth of the fungi.

During each isolation yeast flora of pine needles was also considered.

For the assessment of surface mycoflora of needles, dilution plate method was followed. Dextrose peptone agar containing 1/30009 rose bengal and 30 Mg/lit. streptomycin (Martin, 1950) was used for the isolation of microfungi and yeast. Czapek's dox agar and Malt extract

agar media were also tried but Martin's rose bengal agar medium was found to be more suitable. Composition of the medium is described in the materials and methods (Chapter 1).

Before preparation of the dilution series the pine needles were cut into small pieces with the help of sterilized scissors and foreceps in a laminar flow chamber to avoid contamination and were mixed thoroughly from replicate samples. In each case one g (on wet weight basis) sample was taken in a 250 ml sterilized conical flask containing 100 ml sterilized distilled water. Flasks were shaken vigorously for 30 minutes by hand to detach the surface propagules. Ten ml of the suspension was rapidly transferred in another 250 ml sterilized conical flask containing 90 ml sterilized distilled water with the help of a sterilized 10 ml pippete. Dilution of 1:1000 was found to be suitable for the isolation of mycoflora of pine needles. Separate sterilized pippets were used in preparing each dilution. 0.5 ml of 1:1000 diluted suspension was transferred with the help of a sterilized pippet on solid agar media. The suspension was gently spread uniformly over the surface of the media. Three replicate plates were inoculated in each case and the plates were incubated in a BOD incubator at 25°C for a period of 4-5 days. Fungal colonies including yeasts growing on to the agar were recorded with the help of a digital colony counter and the total number of fungi including yeast was calculated on the basis of dry

weight of the sample taking dilution factor into consideration. Identification of microfungi and yeast upto generic level and wherever possible upto species level was done following the manual of Gilman (1956), Barnett (1972) and Subramanian (1971).

For the isolation of internal colonizers, five pieces of pine needles for each sample were surface sterilized in mercuric chloride solution (1/10000 with 5% industrial alcohol) for 1 min. (Hering, 1965). The pieces were then thoroughly washed several times using sterilized distilled water to remove all traces of mercuric chloride and plated on to the Martin's rose Bengal agar. The plates were incubated at 25°C in a BOD incubator for a period of 3-4 days after which the pieces of the needles were removed and teased gently with the help of a sterilized forceps on a sterilized glass slide and were stained with lactophenol cottonblue and observed under high resolution of microscopic field. In each case five pieces were examined to observe internal fungal colonies . Counts were made on the basis of various fungal and mycelial growth in the needles and their colonies on the medium were identified.

The results of the dilution plate method were represented in terms of percentage relative abundance and percentage frequency of fungi observed or isolated. The result of surface sterilized needles were recorded in terms of percentage frequency only (Vittal, 1976).

Unsterilized leaf pieces were also incubated in

sterilized moist chamber at 25°C for 2-3 weeks for the record of fungi. This practice was followed for 6 months in the beginning but the species isolated were not different from those obtained from by dilution plate method and hence this method was discontinued.

pH and moisture content of the needles - Moisture content and pH of the needles during different samplings were determined by oven dry method and electric pH meter respectively.

Meteriological observation - Atmospheric temperature, rainfall were recorded throughout the investigation.

RESULTS

The experimental findings are presented in the following order -

1. Quantitative assessment of the fungal and yeast population of the phylloplane of green and senescent pine needles of 1955 and 1970 plantation in different sampling periods are represented in the Figs. 16-17. Statistical analyses of the results are depicted in the Tables 35-42.

2. Records of the percentage relative abundance and percentage frequency of fungal species on green and senescent pine needles of 1955 and 1970 plantations in different sampling periods are given in the Tables 43-46 and Figs.18-19.

3. Quantitative assessment of fungal and yeast population from folded to litter stages of pine needles of 1970 plantation is depicted in the Fig.20.

4. Records of percentage relative abundance, frequency and succession of fungal species on folded to litter stages of pine needles of 1970 plantation is represented in the Tables 47-52. Also the records of internal colonizers of surface sterilized needles at different stages of maturity is given in the Tables 53-58.

5. Records of pH and moisture content of the Pine needles at different stages of maturity is depicted in the table 59.

6. Climatic data in the form of graph is given in the Fig.13.

1. <u>Quantitative assessment of fungal and yeast population of the phylloplane of green and senescent pine needles of 1955 and 1970 plantation</u> :-

The fungal and yeast population on the phylloplane of green pine needles was observed to be always lower than that of the senescent needles, and this trend in the population was similar for both the plantations (Figs.16 and 17). The fungal and yeast population of the senescent pine needles increased gradually in both the plantations. In 1955 plantation the fungal and yeast population at the senescent stage was minimum in the beginning i.e. in the month of November and gradually the fungal population increased and attained its peak in the month of July whereas yeast attained the peak in the month of May. Almost similar result was observed for 1970 plantation, where the fungal and yeast population of the senescent pine needles was also observed to be minimum in the beginning i.e. in the month of November. Fungal and yeast population gradually, increased and attained its peak in the month of May.

The fungal population on the phylloplane of green needles of 1955 plantation was also observed to be minimum in the beginning i.e.in the month of November. The population of both Micro fungi and yeast increased gradually and attained the peak in the months of July and May respectively (Fig.16). Almost similar result was observed for 1970 plantation too, except that the peak

in the population of fungi and yeast on the green needles was found to be in the month of September and May respectively (Fig.17).

Statistically no significant variations could be observed in the fungal and yeast population of green and senescent pine needles between two plantations and between different seasons (Tables 35, 36, 39, 40). There was, however, a significant variation in the phylloplane fungal population between green and senescent pine needles of 1955 plantation (Table 37). This variation in the phylloplane fungal population between green and senescent pine needles of 1970 plantation was observed to be statistically not significant (Table 38). In both the plantations statistically there was no significant effect of seasons on the phylloplane yeast and fungal population of green and senescent needles.

2. Percentage relative abundance and frequency of fungal species on green and senescent needles :-

Fourteen species, viz. one phycomycetes, nine fungi imperfecti, two sterile mycelia and two yeasts were isolated from the phylloplane of senescent pine needles of 1955 plantation (Table 43) whereas only nine species were isolated from the phylloplane of green needles (Table 44).

Penicillium chrysogenum, *Sporobolomyces roseus*, *Cladosporium herbarum* and sterile white mycelia were dominant species on phylloplane of senescent pine

needles of 1955 plantation (Table 43).

It was observed that percentage relative abundance of Sporobolomyces roseus on the senescent needles was higher than any other species recorded and highest relative abundance of this species was recorded in the month of July (Fig.18). Though Penicillium chrysogenum, Cladosporium herbarum and sterile white mycelia were recorded as dominant fungal species on senescent needles yet their relative abundance was very low. Highest number of fungal species on senescent needles was recorded in the month of November and September (Table 43). Mucor hiemalis, Aspergillus fumigatus, Geotrichum sp, Alternaria tenuis and Verticillium sp occured as rare phylloplane mycoflora on senescent needles (Table 43).

Fungal species recorded from the green pine needles of the same plantation were Penicillium chrysogenum and sterile white mycelia as rare, Sporobolomyces roseus and Cladosporium herbarum as frequent and common species respectively (Table 44).

It was observed that Sporobolomyces roseus and Aureobasidium pullulans appeared with high relative abundance than any other fungal species on green needles (Table 44 and Fig.18).

Nine fungal species were isolated from the phylloplane of green and thirteen species from senescent pine needles of 1970 plantation (Tables 46,45). Cladosporium herbarum and sterile white mycelia were

recorded as dominant fungi of both the green and senescent needles. From the senescent needles highest number of fungal species was isolated in the month of March whereas July and September yielded the highest number of fungi on the green needles (Tables 46,45 and Fig.19).

3. Quantitative assessment of fungal and yeast population of pine needles from folded to senescent to litter stages :-

During the two years period of studies it has been observed that the microfungal and yeast population of the phylloplane of pine needles increased gradually from folded (bud stage) to senescent and to those which entered into litter stage (Fig.20). The peak of both microfungi and yeast was attained at the litter stage (Fig.20). The least microfungal population was observed on the needles at the bud or folded stage soon after flushing, whereas the least population of yeast was noted at unfolded stage. It was also observed that the total number of microfungi on pine needles at its different stages of growth fluctuated with increase in the population of yeast.

The moisture content of pine needles at different stages of maturity varied. More than seventy percent moisture content in the needle was observed during its young stage (Fig.20). During senescent stage the moisture content abruptly dropped even below ten percent but it increased again when the needles

entered into litter stages (Fig.20). It has also been observed that the microfungal and yeast population of the phylloplane of pine needles at different stages of maturity responded directly to the moisture content of the needles (Fig.20).

4. Records of percentage relative abundance, frequency and pattern of succession of fungi on pine needles from folded to litter stages :-

a) Percentage relative abundance and frequency of fungi :-

Only seven fungal species viz., one ascomycetes, three deuteromycetes, one sterile white mycelia, and two yeast species were isolated from the phylloplane of needles at the bud stage (Table 47). Sporobolomyces roseus and Penicillium chrysogenum were found to be the dominant (Table 47). Sporobolomyces roseus, Aureobasidium pullulans and Geotrichum sp were commonly found to be very active at this stage.It was observed that during each sampling of folded needles (buds) the percentage relative abundance of Sporobolomyces roseus was higher than any other species. The relative abundance of Aureobasidium pullulans and Penicillium chrysogenum was also high during different samplings of folded needles (Table 47). Cladosporium herbarum another very commonly occuring fungus of this region was found to be a rare species on the needles at bud stage. The relative abundance of sterile white mycelia a unidentified fungus was also higher than many other fungal species.

Seven fungal species viz., four members of deuteromycetes, one sterile white mycelia, and two yeasts were isolated from the phylloplane of unfolded pine needles (Table 48). Sporobolomyces roseus, Penicillium chrysogenum, and a sterile white mycelia were found to be dominant on the unfolded needles (i.e. the needles just opened from a outer sheath) (Table 48). Sporobolomyces roseus, Aureobasidium pullulans, Penicillium chrysogenum and a sterile white mycelia (?) were very active on the unfolded pine needles. The relative abundance of Sporobolomyces roseus, Penicillium chrysogenum was found to be higher than any other fungal species. The relative abundance of sterile white mycelia was fairly high than the rest of the fungi isolated (Table 48).

Only six fungal species, viz., three members of deuteromycetes, one sterile white mycelia and two yeasts were isolated from the young expanding green needles (Table 49). Cladosporium herbarum, Penicillium chrysogenum, and sterile white mycelia were found to be the dominant on the phylloplane of young expanding green needles. The activities of yeast species were quite less at this stage (Table 49). Aureobasidium pullulans was found to be rare at this stage. The relative abundance of Cladosporium herbarum and sterile white mycelia during different samplings was higher than the other fungal species (Table 49).

Penicillium chrysogenum though was recorded as a dominant fungus on young expanding green pine needles its relative abundance was very low. Pleochaeta setosa a deuteromycetes was very common fungus on the needles at this stage.

Nine fungal species, viz., one phycomycetes, five members of deuteromycetes, one sterile white mycelia and two yeasts were isolated from the phylloplane of matured green needles (Table 50). Cladosporium herbarum and sterile white mycelia were the dominant fungi. Sporobolomyces roseus, and Aureobasidium pullulans though were not dominant on matured green needles yet their percentage relative abundance was fairly high. The relative abundance of Cladosporium herbarum and sterile white mycelia was also high on the phylloplane of matured green needles (Table 50).

It was observed that the total number of fungal species increased on the phylloplane of needles when the latter entered into senescent stage (Table 51). Ten fungal species, viz., one phycomycetes, one sterile white mycelia, two yeast species and six fungi imperfecti were isolated from the phylloplane of senescent needles. Cladosporium herbarum and sterile white mycelia were found to be dominant at this stage. Many species like Penicillium chrysogenum, Trichoderma viride, Fusarium sp, Verticillium sp and a few others were recorded as rare fungi on the senescent needles

(Table 51).

The maximum number of species was recorded when the needles entered into litter stage. Thirteen fungal species, viz., one phycomycetes, one ascomycetes, one sterile white mycelia, two yeast species and eight fungi imperfecti were isolated. The activity of Sporobolomyces roseus was very high during the litter stage (Table 52). Sporobolomyces roseus and Cladosporium herbarum were the dominants. Sporobolomyces roseus showed the highest relative abundance on the needles at this stage (Table 52). Many soil fungi like Trichoderma viride, Fusarium sporotrichoides and Phoma humicola appeared on the litter.

b) Internal fungal colonizers of pine needles from folded to litter stages :-

It was observed from this experiment that the internal fungal colonists were very much different from the phylloplane of pine needles. One sterile white mycelia was always recorded internally from folded to litter stages (Tables 53-58). Sporobolomyces roseus, sterile white mycelia and Pleochaeta setosa were found to be dominant internally at bud stage. The former two fungal species were dominant internally at unfolded stage also. Papullaria was also a frequently occuring species internally at this stage. Sterile white mycelia was dominant as an internal fungus in young expanding green needles. Pleochaeta setosa and Stemphyllium sp. were recorded as frequent and common species

respectively. Alternaria tenuis and Aspergillus fumigatus were associated internally with the needles when it reached at matured green stage. Sterile white mycelia was found to be dominant internally at this stage. When pine needles entered into senescent and litter stages more fungal species were noted to be present internally. Sterile white mycelia was isolated as dominant fungi internally from senescent to litter stages of the needles.

c) Pattern of succession of fungi on pine needles from folding to litter stages :-

A gradual transition in the quality of phylloplane fungi occured from the bud to senescent to litter stages of the needles.

Penicillium chrysogenum, sterile white mycelia and Sporobolomyces roseus were the dominant forms immediately after flushing (Tables 46,47). When the needles were undergoing maturation and entered into the senescent stage Cladosporium herbarum and sterile white mycelia outnumbered Penicillium chrysogenum and and Sporobolomyces roseus and they became the dominant forms (Tables 48-50).

At the latter stage Sporobolomyces roseus and Cladosporium herbarum outnumbered sterile white mycelia and they dominated the phylloplane of leaf litter. Other forms like Trichoderma viride, Phoma humicola and Fusarium sporotrichoides appeared during the latter stage (Table 51).

Figure - 16 : Seasonal variation in microfungal and yeast population on attached senescent and green needles collected from selected pine trees of 1955 plantation.

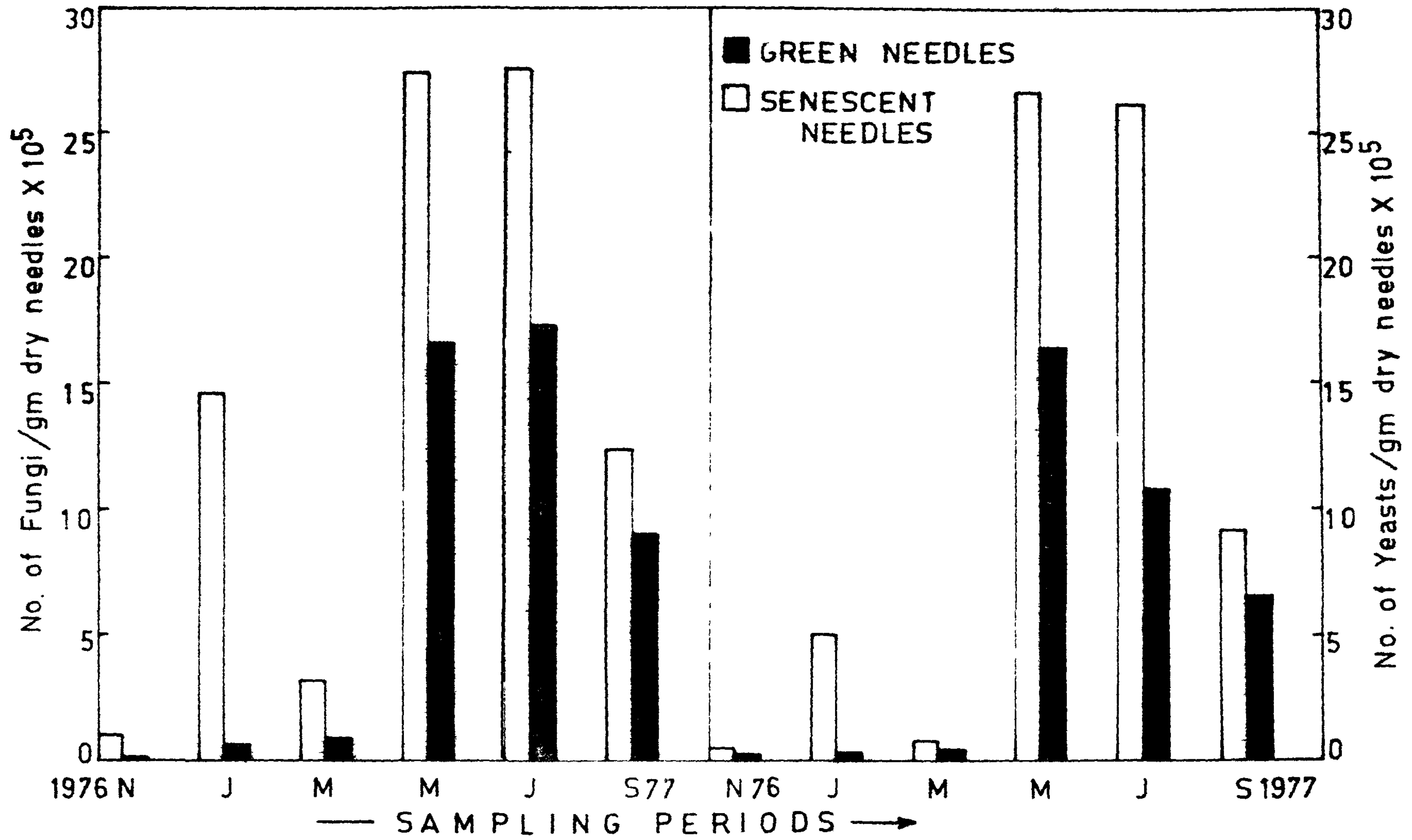


Figure 16

Figure - 17 : Seasonal variation in microfungal and yeast population on attached senescent and green pine needles collected from selected pine trees of 1970 plantation.

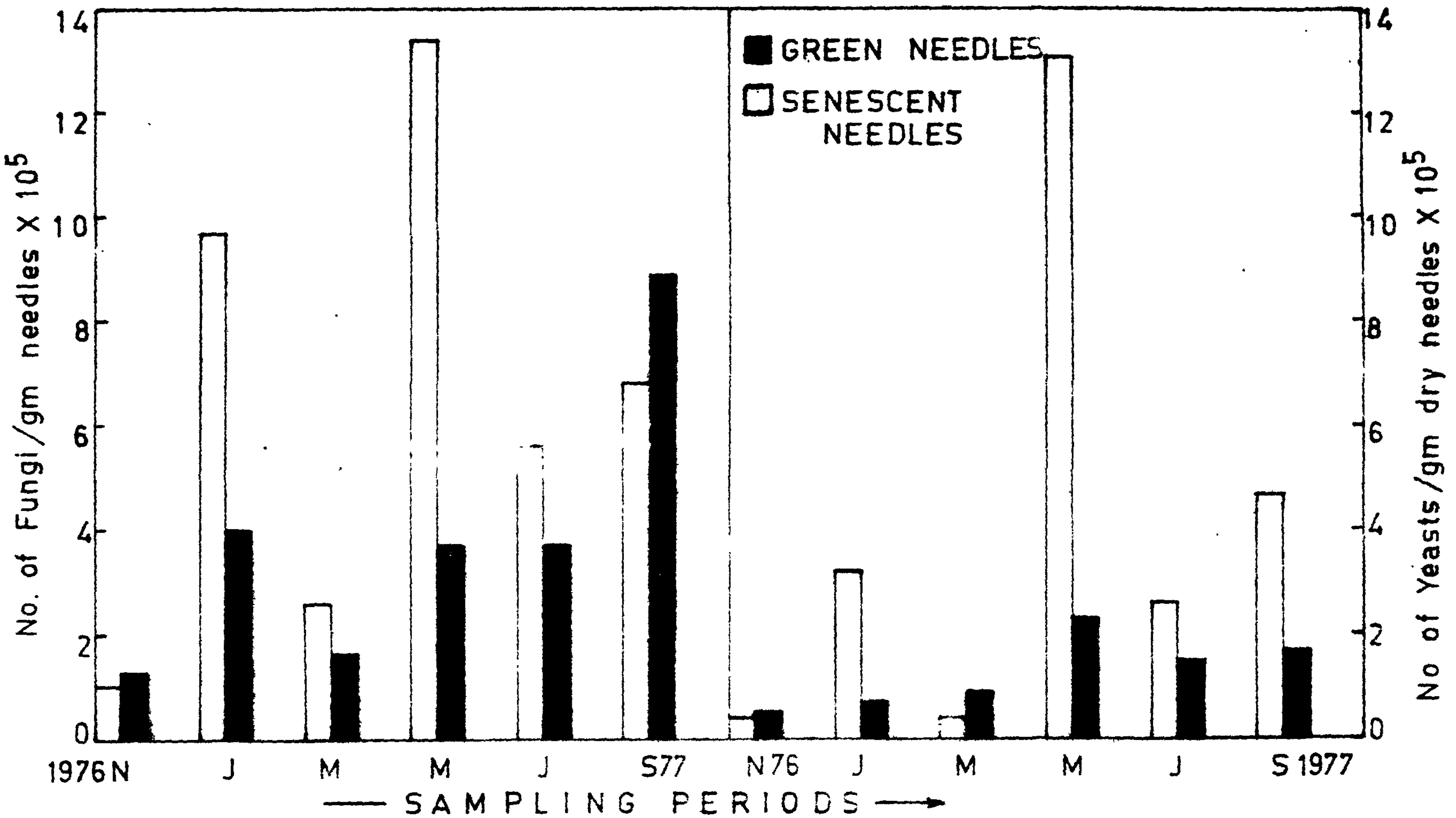


Figure 17

Figure - 18 : Seasonal variation in percentage relative abundance of different fungal species occured on green and senescent pine needles of 1955 plantation.

1 = *Mucor hiemalis*, 2 = Unidentified sphaeropsidales, 3 = *Chaetomella* sp., 4 = *Geotrichum* sp., 5 = *Trichoderma viride*, 6 = *Aspergillus fumigatus*, 7 = *Penicillium chrysogenum*, 8 = *Verticillium* sp., 9 = *Cladosporium herbarum*, 10 = *Alternaria tenuis*, 11 = Sterile white mycelia, 12 = Sterile black mycelia, 13 = *Sporobolomyces roseus*, 14 = *Aureobasidium pullulans*.

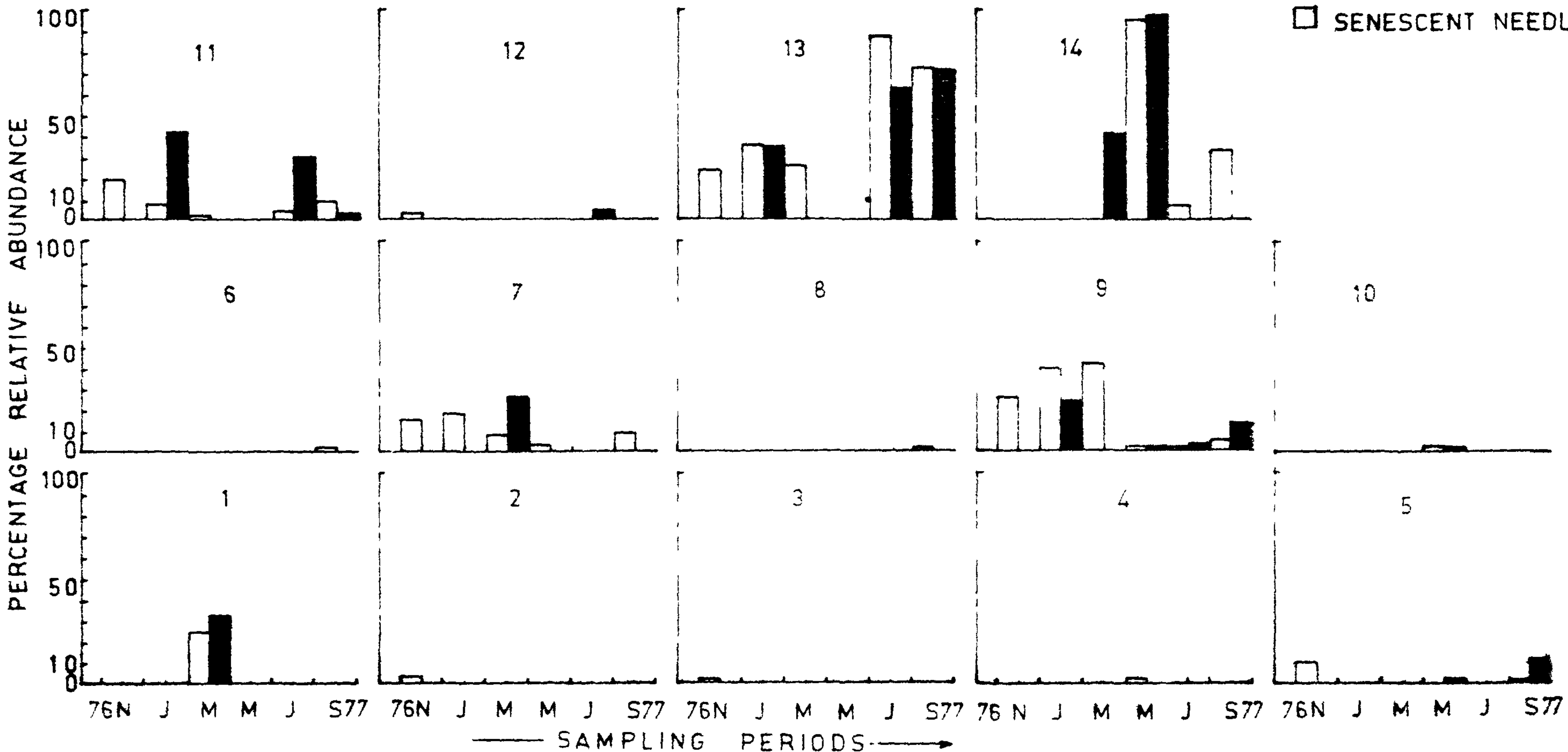


Figure 18

Figure - 19 : Seasonal variation in the percentage relative abundance of different fungal species occured on green and senescent pine needles of 1970 plantation.

1 = *Mucor hiemalis*, 2 = *Trichoderma viride*, 3 = *Penicillium chrysogenum* 4 = *Cladosporium herbarum*, 5 = *Alternaria tenuis*, 6 = *Fusarium sporotrichoides*, 7 = *Sporobolomyces roseus*, 8 = *Aureobasidium pullulans*, 9 = Sterile white mycelia.

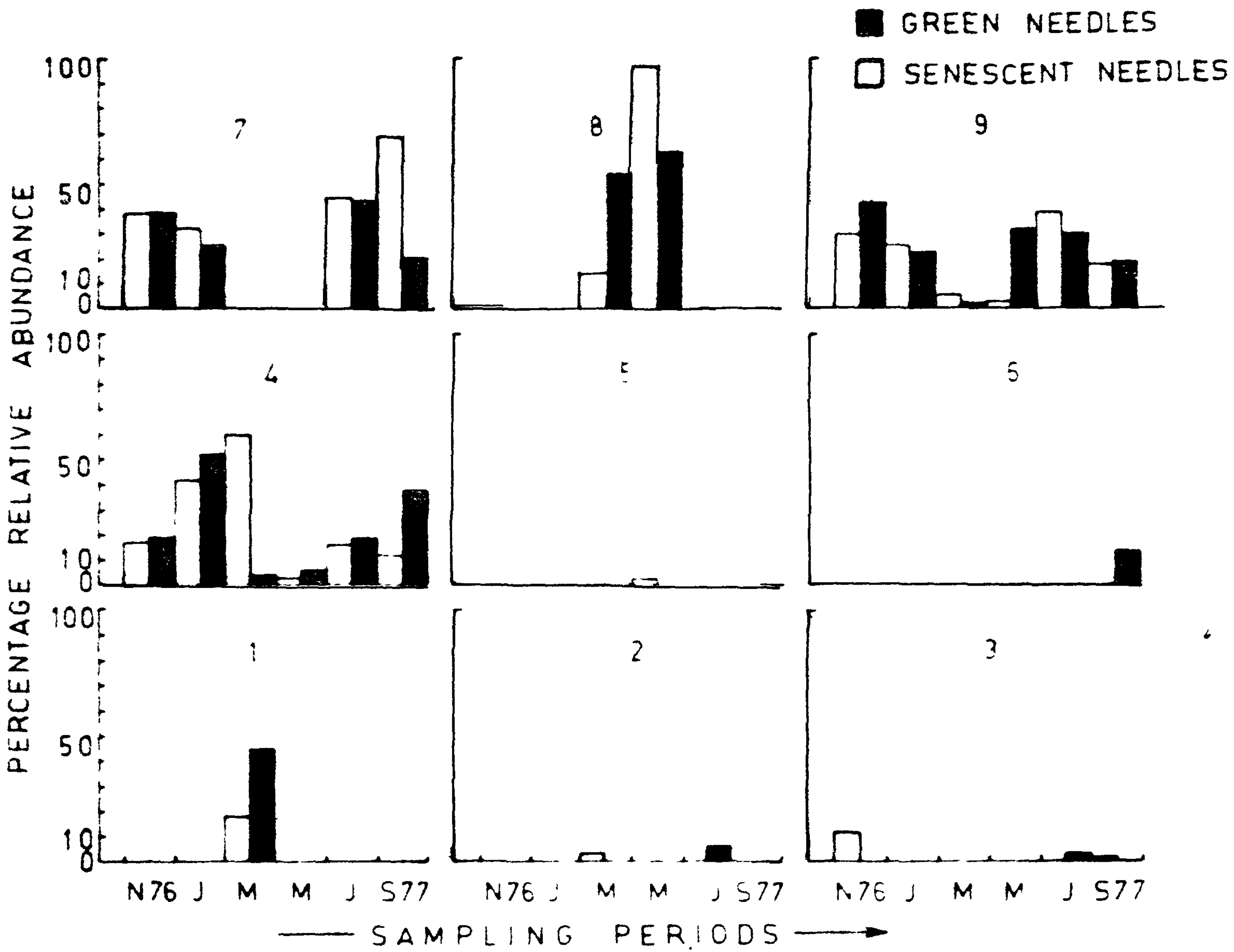


Figure 19

Figure - 20 : Histograms showing seasonal variation in fungal and yeast population on pine needles during different stages of maturation (Each point represents mean of five samplings).

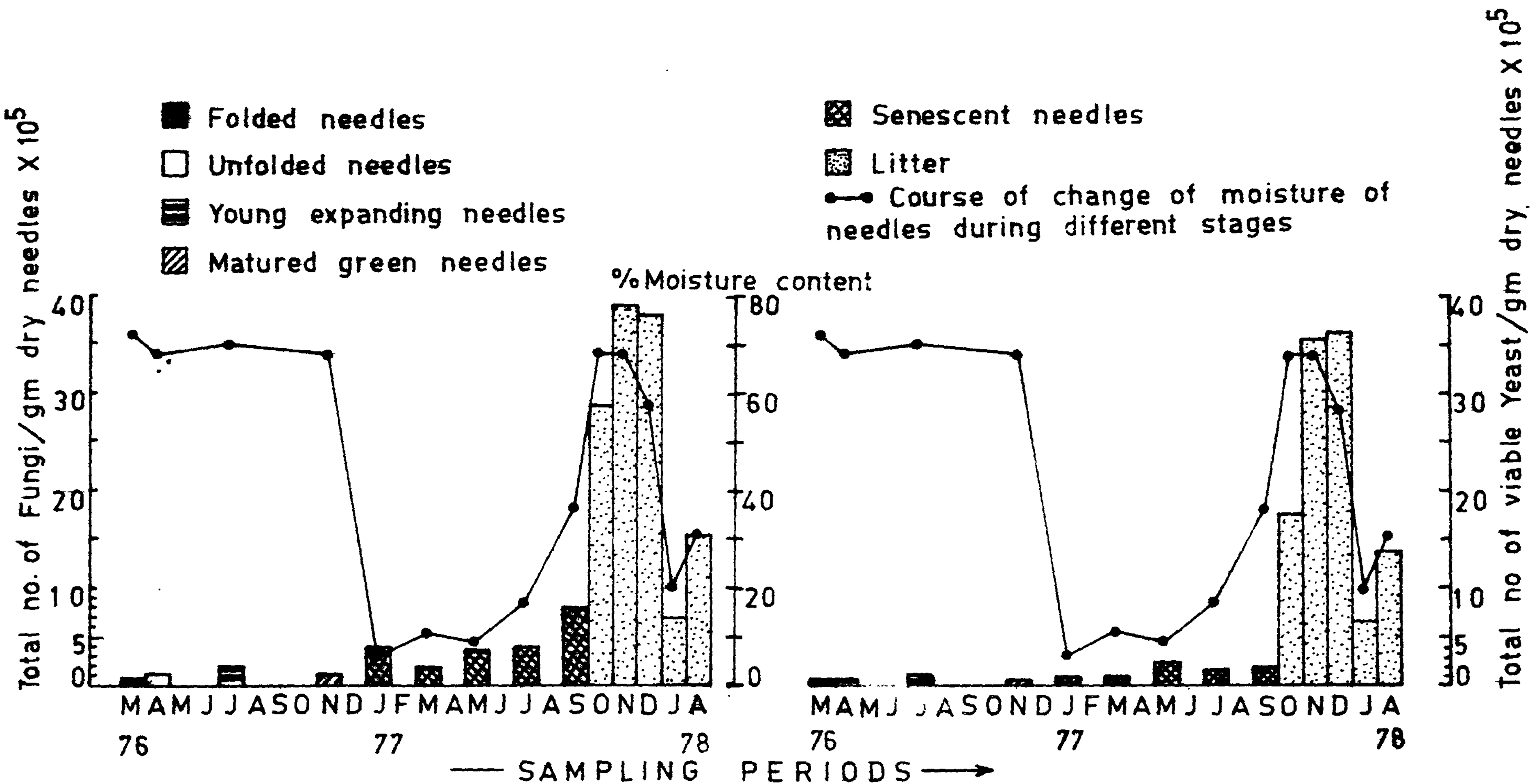


Figure 20

Table - 35. Analysis of variance for fungal population of green pine needles under two plantations and in different months.

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value 5%	Remarks
Between plantations	1	36.92	36.92	1.28	6.61	Not significant
Between sampling periods	5	219.22	43.84	1.52	5.05	Not significant
Error	5	143.82	28.76			
Total	11					

The above table reveals that the variation in the fungal population of green pine needles between two different plantations and between different sampling periods is statistically not significant.

Table - 36. Analysis of variance for fungal population of senescent pine needles under two plantations and in different months.

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value 5%	Remarks
Between plantations	1	184.71	184.71	5.03	6.61	Not significant
Between sampling periods	5	571.33	114.26	3.11	5.05	Not significant
Error	5	183.38	36.67			
Total	11					

The above table reveals that the variation in the fungal population of senescent pine needles between two plantations and between different sampling periods is statistically not significant.

Table - 37. Analysis of variance for fungal population between green and senescent pine needles of 1955 plantation.

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value 5%	Remarks
Between needles	1	145.95	145.95	10.01	6.61	Significant
Between sampling periods	5	904.12	180.82	12.41	5.05	Significant
Error	5	72.87	14.57			
Total	11					

The above table reveals that the variation in the fungal population between green and senescent pine needles and between sampling periods is statistically significant.

Table - 38. Analysis of variance for fungal population between green and senescent pine needles of 1970 plantation.

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value 5%	Remarks
Between needles	1	20.85	20.85	2.25	6.61	Not significant
Between sampling periods	5	94.61	18.92	2.04	5.05	Not significant
Error	5	46.15	9.23			
Total	11					

The above table reveals that the variation in the fungal population between green and senescent pine needles and between different sampling periods is statistically not significant.

Table - 39. Analysis of variance for yeast population of green pine needles under two plantations and in different months.

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value 5%	Remarks
Between plantations	1	58.96	58.96	3.14	6.61	Not significant
Between sampling periods	5	139.76	27.95	1.48	5.05	Not significant
Error	5	93.85	18.77			
Total	11					

The above table reveals that the variation in the yeast population of green pine needles between two plantations and between different sampling periods is statistically not significant.

Table - 40. Analysis of variance for yeast population of senescent pine needles under two plantations and in different months.

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value 5%	Remarks
Between plantations	1	160.46	160.46	3.61	6.61	Not significant
Between sampling periods	5	624.84	124.96	2.81	5.05	Not significant
Error	5	222.13	44.42			
Total	11					

The above table reveals that the variation in the yeast population of senescent pine needles between two plantations and between different sampling periods is statistically not significant.

Table - 41. Analysis of variance for yeast population between green and senescent pine needles of 1955 plantation.

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value 5%	Remarks
Between needles	1	95.77	95.77	5.21	6.61	Not significant
Between sampling periods	5	873.19	174.63	9.51	5.05	Significant
Error	5	91.77	18.35			
Total	11					

The above table reveals that the variation in the yeast population between green and senescent pine needles is statistically not significant but it significantly varies between different sampling periods.

Table - 42. Analysis of variance for yeast population between green and senescent pine needles of 1970 plantation.

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value 5%	Remarks
Between needles	1	23.02	23.02	2.64	6.61	Not significant
Between sampling periods	5	72.06	14.41	1.65	5.05	Not significant
Error	5	43.55	8.71			
Total	11					

The above table reveals that the variation in the yeast population between green and senescent pine needles and between different sampling periods is statistically not significant.

Table - 43. Percentage relative abundance and frequency of fungal species on senescent pine needles of 1955 plantation.

Fungal species isolated	Percentage relative abundance						Frequency
	Nov 1976	Jan 1977	Mar	May	Jul	Sep	
Mucor hiemalis	-	-	24.0	-	-	-	R
Unidentified Sphaeropsidales	2.0	-	-	-	-	-	R
Chaetomella sp	2.0	-	-	-	-	-	R
Geotrichum sp	-	-	-	1.0	-	-	R
Trichoderma viride	10.0	-	-	-	-	2.0	O
Aspergillus fumigatus	-	-	-	-	-	1.0	R
Penicillium chrysogenum	15.0	18.0	8.0	1.0	-	9.0	D
Verticillium sp	-	-	-	-	-	1.0	R
Cladosporium herbarum	26.0	40.0	41.0	1.0	1.0	5.0	D
Alternaria tenuis	-	-	-	1.0	-	-	R
Sterile white mycelia	19.0	7.0	1.0	-	4.0	9.0	D
Sterile black mycelia	2.0	-	-	-	-	-	R
Sporobolomyces roseus	24.0	35.0	26.0	-	88.0	73.0	D
Aureobasidium pullulans	-	-	-	96.0	7.0	33.0	F

D = Dominant (81 - 100% Frequency) R = Rare(1-20% Frequency)
F = Frequent (41 - 60% Frequency)
O = Occasional (21 - 40% Frequency)

Table - 44. Percentage relative abundance and frequency of fungal species on green pine needles of 1955 plantation.

Fungal species isolated	Percentage relative abundance					Frequency
	Jan 1977	Mar	May	Jul	Sept.	
Mucor hiemalis	-	32.0	-	-	-	R
Trichoderma viride	-	-	1.0	-	11.0	O
Penicillium chrysogenum	-	26.0	-	-	-	R
Cladosporium herbarum	24.0	-	1.0	3.0	14.0	C
Alternaria tenuis	-	-	1.0	-	-	R
Sterile white mycelia	41.0	-	-	30.0	3.0	F
Sterile black mycelia	-	-	-	4.0	-	R
Sporobolomyces roseus	35.0	-	-	63.0	72.0	F
Aureobasidium pullulans	-	42.0	97.0	-	-	O

<u>Table - 45.</u> Percentage relative abundance and frequency of fungal species on senescent pine needles of 1970 plantation.

Fungal species isolated	Percentage relative abundance						%Frequency
	NOV 1976	JAN 1977	MAR	MAY	JUL	SEP	
Mucor hiemalis	-	-	17.0	-	-	-	R
Trichoderma viride	-	-	2.0	-	-	-	R
Aspergillus fumigatus	3.0	-	1.0	-	-	-	O
Penicillium chrysogenum	11.0	-	-	-	-	2.0	O
Cladosporium herbarum	17.0	40.0	57.0	1.0	15.0	12.0	D
Alternaria tenuis	-	-	-	1.0	-	-	R
Alternaria alternata	2.0	-	-	-	3.0	-	O
Chaetomella sp	-	-	3.0	2.0	-	-	O
Sterile white mycelia	28.0	23.0	6.0	1.0	39.0	17.0	D
Sterile black mycelia	-	2	4	-	-	-	O
Sporobolomyces roseus	37.0	32.0	-	-	43.0	69.0	C
Aureobasidium pullulans	-	-	10.0	95.0	-	-	O
Geotrichum sp.	2.0	3.0	-	-	-	-	O

Table - 46. Percentage relative abundance and frequency of fungal species on green pine needles of 1970 plantation.

Fungal species isolated	Percentage relative abundance						Frequency
	NOV 1976	JAN 1977	MAR	MAY	JUL	SEP	
Mucor hiemalis	-	-	45.0	-	-	-	R
Trichoderma viride	-	-	-	-	6.0	-	R
Penicillium chrysogenum	-	-	-	-	3.0	-	R
Verticillium sp	-	-	-	-	-	10.0	R
Cladosporium herbarum	19.0	52.0	1.0	6.0	18.0	38.0	D
Fusarium sp	-	-	-	-	-	14.0	R
Sterile white mycelia	43.0	23.0	1.0	31.0	30.0	18.0	D
Sporobolomyces roseus	38.0	25.0	-	-	43.0	20.0	C
Aureobasidium pullulans	-	-	53.0	63.0	-	-	O

Table - 47. Percentage relative abundance and frequency of fungal species on folded pine needles (Buds).

Fungal species isolated	Percentage relative abundance					Frequency
	Sample 1	Sample 2	Sample 3	Sample 4	Sample5	
Unidentified ascomycetes	12.50	-	-	12.20	-	O
Penicillium chrysogenum	12.50	29.85	33.92	29.24	18.51	D
Cladosporium herbarum	6.26	-	-	-	-	R
Sterile white mycelia	25.0	-	-	24.40	-	O
Sporobolomyces roseus	34.39	44.78	35.72	21.96	74.07	D
Aureobasidium pullulans	3.10	14.95	30.36	12.20	-	C
Geotrichum sp.	6.25	10.44	-	-	7.42	F

Table - 48. Percentage relative abundance and frequency of fungal species on unfolded pine needles.

Fungal species isolated	Percentage relative abundance					Frequency
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	
Aspergillus fumigatus	1.63	-	-	-	-	R
Penicillium chrysogenum	59.02	43.51	53.86	19.34	6.25	D
Papullaria sp	1.66	-	-	-	-	R
Sterile white mycelia	4.91	14.49	23.08	8.06	25.00	D
Sporobolomyces roseus	16.39	14.49	15.38	72.06	50.01	D
Aureobasidium pullulans	16.39	21.73	7.68	-	18.84	C
Geotrichum sp	-	5.78	-	-	-	R

Table - 49. Percentage relative abundance and frequency of fungal species on young expanding green pine needles.

Fungal species isolated	Percentage relative abundance					Frequency
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	
Penicillium chrysogenum	6.55	17.04	16.12	6.86	7.69	D
Pleochaeta setosa	9.34	3.42	3.25	-	7.69	C
Cladosporium herbarum	28.04	34.09	48.38	63.74	30.77	D
Sterile white mycelia	23.36	39.77	32.25	14.70	53.85	D
Sporobolomyces roseus	28.04	5.68	-	14.70	-	F
Aureobasidium pullulans	4.67	-	-	-	-	R

Table - 50. Percentage relative abundance and frequency of fungal species on matured green pine needles.

Fungal species isolated	Percentage relative abundance					Frequency
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	
Mucor hiemalis	-	-	17.0	-	-	R
Trichoderma viride	-	-	2.0	-	-	R
Aspergillus fumigatus	3.0	-	1.0	-	-	O
Penicillium chrysogenum	11.0	-	-	-	-	R
Cladosporium herbarum	17.0	42.0	60.0	1.0	15.0	D
Alternaria tenuis	-	-	-	1.0	-	R
Sterile white mycelia	30.0	26.0	6.0	1.0	39.0	D
Sporobolomyces roseus	39.0	32.0	-	-	46.0	F
Aureobasidium pullulans	-	-	14.0	97.0	-	O

Table - 51. Percentage relative abundance and frequency of fungal species on senescent pine needles.

Fungal species isolated	Percentage relative abundance					Frequency
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	
Mucor hiemalis	-	45.0	-	-	-	R
Trichoderma viride	-	-	-	6.0	-	R
Penicillium chrysogenum	-	-	-	1.0	-	R
Verticillium sp	[illegible]	[illegible]	[illegible]	[illegible]	10.0	R
Cladosporium herbarum	52.0	1.0	6.0	18.0	38.0	D
Fusarium sp	-	-	-	-	14.0	R
Sterile white mycelia	23.0	1.0	31.0	30.0	18.0	D
Unidentified colony	-	-	-	2.0	-	R
Sporobolomyces roseus	25.0	-	-	43.0	20.0	F
Aureobasidium pullulans	-	53.0	63.0	-	-	O

Table - 52. Percentage relative abundance and frequency of fungal species on pine leaf litter.

Fungal species isolated	Percentage relative abundance					Frequency
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	
Mucor hiemalis	-	0.14	-	0.14	-	O
Phoma humicola	0.99	-	-	5.49	-	O
Trichoderma viride	0.99	-	0.28	-	-	O
Penicillium chrysogenum	1.39	1.20	1.24	-	-	F
Cladosporium herbarum	2.69	0.89	1.21	4.09	5.57	D
Fusarium sporotrichoides	0.39	-	-	-	2.56	O
Pleochaeta setosa	-	0.05	-	-	-	R
Endomyces sp	-	0.10	-	-	-	R
Cylindrocladium sp	-	3.36	-	-	-	R
Sterile white mycelia	6.48	4.20	2.38	7.32	-	C
Unidentified sp ?	0.44	-	-	-	-	R
Sporobolomyces roseus	78.16	90.06	94.89	81.89	91.87	D
Aureobasidium pullulans	8.47	-	-	1.07	-	O

Table - 53. Internal fungal colonizers of surface sterilized folded pine needles (Buds) and their frequency.

Fungal species recorded	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Frequency
Pleochaeta setosa	+	+	+	+	+	D
Sterile white mycelia	+	+	+	+	+	D
Sporobolomyces roseus	+	+	+	+	+	D

D = Dominant (81-100% frequency)

+ = present.

Table - 54. Internal fungal colonizers of surface sterilized unfolded pine needles and their frequency.

Fungal species isolated	SAMPLE 1	SAMPLE 2	SAMPLE 3	SAMPLE 4	SAMPLE 5	Frequency
Papullaria sp	+	-	-	-	+	F
Sterile white mycelia	+	+	+	+	+	D
Sporobolomyces roseus	+	+	+	+	+	D

Table - 55. Internal fungal colonizers of surface sterilized young expanding green pine needles.

Fungal species recorded	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Frequency
Stemphyllium sp	+	-	+	+	+	C
Pleochaeta setosa	-	-	+	+	+	F
Sterile white mycelia	+	+	+	+	+	D

Table - 56. Internal fungal colonizers of surface sterilized matured green pine needles and their frequency.

Fungal species recorded	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Frequency
Aspergillus fumigatus	+	+	-	-	-	O
Stemphyllium sp	+	+	-	+	+	C
Akternaria tenuis	-	-	+	+	+	F
Sterile white mycelia	+	+	+	+	+	D

Table - 57. Internal fungal colonizers of surface sterilized senescent pine needles and their frequency.

Fungal species recorded	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Frequency
Penicillium chrysogenum	+	+	+	+	+	D
Stemphyllium sp	-	+	-	-	+	O
Alternaria tenuis	-	-	+	+	-	O
Pleochaeta setosa	+	+	+	+	+	D
Sterile white mycelia	-	+	+	+	+	C

Table - 58. Internal fungal colonizers of surface sterilized pine leaf litter and their frequency.

Fungal species recorded	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Frequency
Phoma humicola	+	+	+	-	+	C
Stemphyllium sp	+	+	+	-	-	F
Pleochaeta setosa	-	+	-	-	-	R
Sterile white mycelia	+	+	+	+	+	D
Aureobasidium pullulans	+	-	-	+	-	O
Sporobolomyces roseus	+	+	+	+	+	D

\+ = ~~Positive~~ Present

\- = Absent

Table - 59. Moisture content in percentage and pH (in bracket) of pine needles from folded-senescent-litter stages.

Sampling periods / Samples	MAR76 25.3.76	APR 24.4.76	JUL 25.7.76	NOV 25.11.76	JAN77 25.1.77	MAR 25.3.77	MAY 25.5.77	JUL 25.7.77	SEP 25.9.7
Folded needles (Buds)	72.9 (4.0)								
Unfolded needles (Buds opened)		68.8 (3.9)							
Young expanding green needles			70.5 (4.5)						
Matured green needles				68.0 (4.30)					
Senescent needles					6.0 (4.70)	10.5 (4.10)	9.2 (4.70)	17.0 (4.35)	36.5 (4.75)
Litter from soil surface (Needles at litter stages)									

DISCUSSION

A perusal of the figures 16, 17 and 20 suggests that low fungal and yeast population on young green needles may be due to poor nutrient status and production of more antimicrobial substances on the needle surface. Cholodney (1932), Tukey and Morgan (1963) pointed out that although young leaves appear delicate and fragile, they are less susceptible to leaching than are the older leaves. At present no data is available on the nutrient status of the needles of Pinus kesiya at different stages of its growth nor any data is available on the quality and quantity of antimicrobial substances present on the needles at different stages of growth which influence the saprophytic activity on phylloplane. This aspect of phylloplane studies of Pinus kesiya will be worth undertaking in future. Dickinson (1967) also advocated that increased activity of saprophytes on senescent leaves may be primarily a result of alterations in leaf physiology. He also noticed a rapid increase in the fungal and yeast population associated with leaf senescente of Pisum and other leaves. Bailey (1969) has suggested that active phylloplane growth which occurs while leaves senesce may be the result of decrease in phytoalexin production. Leaching of phenolic compounds (anti-fungal substance) from leaf surface have been ruled out by Morgan and Tukey (1964).

di Menna (1971) also observed that numbers of moulds and yeasts/g leaves were greater on litter and senescent leaves than young leaves. Sinha (1971)

observed a similar increase in the microflora on older leaves than young leaves of some agricultural crop plants. Dickinson et al (1976) also observed a large increase in the population of yeasts and filamentous fungi on ageing green leaves of winter wheat. Mishra and Tewari (1976) accounted that amount of sugars and amino acids leached from the leaf surface increase with age of the leaves. In our present study also the senescent needles presumably leached higher amounts of nutrients as it was observed in the litter decomposition studies in the Chapter 3 that senescent pine needles contained higher amount of amino acids and soluble carbo-hydrates, which favoured the growth of higher fungal and yeast population on senescent needles.

Higher counts of fungal and yeast population was observed during summer (May-September) periods on green and senescent needles of both the plantation where as low population was observed during winter season (November-January) (Figs.16,17). This variation in counts of fungi and yeast was mainly due to the fluctuation in the quantity of Cladosporium herbarum, Sporobolomyces roseus and Aureobasidium pullulans during the periods. The fluctuation in atmospheric temperature, rainfall and humidity of the region probably affected the microbial population in the environment (Tables 43-46 and Fig.13). Atmospheric temperature, rainfall and humidity of the region had profound effects on the normal distribution of the

airspora (Mishra, 1971). Ruscoe (1971) also observed a peak in fungal activity in summer on living and dead leaves of Nothofagus truncata. An occasional higher counts in the fungal population on green and senescent needles was recorded in January due to the increase in the population of Cladosporium herbarum and Sporobolomyces roseus during that period (Figs.16,17,18,19). Mishra and Srivastava (1970) also recorded that Cladosporium was abundantly present on the living and dead leaves of the plants during winter season.

The studies on the phylloplane of pine needles from folded (buds) to litter stage suggests that the higher population of fungi and yeasts on litter may be due to the removal of waxy materials present on the pine needles by the action of higher population of yeasts (Fig.20). Low population on the folded (buds) needles to young matured needles was perhaps due to the presence of thick waxy cuticle on the needles (Fig.20). Waxes have been shown to contain antifungal compounds as demonstrated by Blakeman et al (1973), which increase the resistance of the younger needles to fungal pathogens and affect the leaching of nutrients.

An increase in moisture content of the needles at litter stage even above 60% level, favoured the growth of saprophytic fungi and yeasts on the decomposing litter. Increase in moisture content, however, did not favour the growth of fungi and yeasts

on young needles (Fig.20). This suggests that young actively growing tissue is relatively immune to leaching of mineral nutrients, carbohydrates and aminoacids on the surface due to the hydrophobic nature (Tukey 1971), which makes the young needles as poor habitat for microbes. This hydrophoby of young needles decreases with the maturity of needles. Mature tissue approaching senescence is very susceptible to leaching (Schoch, 1955).Such leaves with increased nutrient status are more favourable habitat for the growth of the microbes. The higher moisture content of the (moribound needles) litter (Table 59, Fig.20) encouraged the leaching of nutrients on the surface of the needles which favoured the growth of fungal and yeast population vigorously.

pH of the needles which varied from folded to litter stage (Table 59) did not affect much the pattern of colonization of pine needles by different microfungi.

The occurence of more fungal species on senescent needles which enter into litter stage suggests that by the time the needles fall on the surface of the forest floor they are substantially colonized by a variety of saprophytic and parasitic fungi (Tables 43, 45,51,52,57,58). Leaves of Pinus sylvestris (Kendrick and Burges, 1962), Fagus sylvatica (Hogg and Hudson, 1966) and Eucalyptus regnans (Macauley and Thrower,1966),

have all been found to be colonized by a variety of parasitic and saprophytic fungi prior to leaf fall.

Sporobolomyces roseus was isolated with relatively higher population from senescent and litter leaves and also during early stage of needles (folded to unfolded stage) immediately after flushing (Tables 43,45,47,48,52) (Figs.18,19). This indicates that the organism is a primary colonizer of pine needles, whose growth on phylloplane increase with increase of the age of the needles.

The increase in the population of *Sporobolomyces roseus* on senescent and green needles during summer (May-September) may be correlated with the increase in the atmospheric temperature of the locality as well as its probable increase in air during this periods. This sharp increase in numbers of *S. roseus* has been observed on the leaves of several plants (Last, 1955; di Menna,1959; Pugh and Buckley,1971 a,b). This has been attributed to the rise in temperature (di Menna,1959) and has also been correlated with leaf age and numbers of *S. roseus* in the air spora at that time (Last,1955).

The presence of *Cladosporium herbarum* with fairly high frequency on pine needles at all stages immediately after flushing specially on senescent needles suggests that it is an active colonizer of pine needles (Tables 43-52). Senescent needles encourage more luxuriant growth of *Cladosporium*. Kerling (1964)

also observed an increase of Cladosporium sp. on senescent rye leaves. Cladosporium herbarum has been isolated previously from leaves of all ages from apple (Hislop and Cox, 1969), beech (Hogg and Hudson 1966) and birch, hazel and ash (Hering 1965). The higher population of Cladosporium herbarum on both green and senescent needles during winter season (November-January) reveals that lower atmospheric temperature, scanty rainfall and presence of higher amount of dew on leaf surface probably favoured the growth of this species during winter (Figs.18,19). Mishra and Srivastava (1970) also observed that Cladosporium herbarum was abundantly present on the living and dead leaves of the plants during winter season in Gorakhpur.

Aureobasidium pullulans has been isolated from very young needles (folded and unfolded) and it has actually colonized the phylloplane of young green needles (Tables 44,46,47,48). It formed vigorously growing colonies whose development declined on litter after leaf fall (Table 52). A. pullulans has been isolated from very young tissues (unfolded leaves) by other workers (Hudson and Webster, 1958; Smith and Wieringa, 1953). Ruscoe (1971) cultured A. pullulans from the phylloplane of very young leaves of Nothofagus truncata and observed vigorously growing colonies whose development increased with increasing leaf age and then declined at leaf fall. Godfrey (1974)

isolated *A. pullulans* on *Pteridium aquilinum* from June and Last *et al* (1972) observed abundance of *A. pullulans* on green leaves from July. In the present study, however, the incidence of *A. pullulans* on both senescent and green pine needles was recorded from the month of March, and the population attained a peak in May and declined thereafter (Figs.18,19). The peak population of this species in the month of May, may be explained in terms of high growth rates induced by high ambient temperature and sufficient moisture in the atmosphere. The fall in population after May is probably due to high rainfall during June-July which washed down the fungus from the leaf surface (Fig.13).

A large number of sterile white mycelia was regularly isolated both internally and externally from the pine needles of all ages immediately after flushing (Tables 53-58 and 43-52). From its high frequency of isolation and occurence it may be assumed that it is one of the important colonizers of pine needles whose growth was not regulated by the age of pine needles. This is because the fungus was also present even after leaf fall and during decomposition of pine needles on forest floor. The variation in its frequency of occurence and population was governed rather by the variation in the atmospheric temperature, rainfall, humidity of the locality. Ruscoe (1971) isolated a large number of sterile hyaline and dark mycelia from living and dead leaves of *Nothofagus*

truncata. From this study it has been revealed that it is one of the important colonizers of Pine needles of this region, whose importance on the phylloplane needs detailed investigation in connection with host parasite relationship, physiology and taxonomy.

Penicillium chrysogenum was observed to be a significant superficial colonist and was present on needles of all ages (Tables 43-52). This suggests that its growth was not supported by the age of the needles but was more profoundly affected by the different environmental factors, which is in agreement with the result of Ruscoe (1971) who also isolated Penicillium Sp. from the leaves of Nothofagus truncata of all ages. Prevalence of this species on different age of the needles reflected the abundance of this species in the airspora during different sampling periods (Gregory and Hirst, 1957).

Other fungi which were recorded both by dilution plate method and surface sterilized needles (Tables 47-52, 53-58) of different ages,occured sporadically and were restricted in the needles of different ages. Somewhat similar restricted occurence of fungi on coniferous needles was reported by Brandsberg (1969).

Chapter - 3

MICROBIAL DECOMPOSITION OF PINE LEAF LITTER.

INTRODUCTION

Plant litter decomposition is one of the most important phenomenon in any forest ecosystems. (Rosswall et al 1975). In order to understand the dynamics of forest ecosystem a number of studies have been made on litter fall, a major pathway for both nutrient and energy transfer (Bray and Gorham, 1964). Several studies indicate that in temperate deciduous forests approximately 70% of the annual uptake of macro-nutrients (N,P,K, Ca and Mg) are returned via litterfall (Duvigneaud et al, 1970). As indicated by Olson (1963), many Tundra ecosystems accumulate organic matter, which is caused by low rates of decomposition. Large amounts of nutrients are thus locked up in the organic matter, and are not available to the plants until mineralized by microorganisms. Therefore, the role of microorganisms in plant litter decomposition is of paramount importance in recycling of the nutrients. The decayed lignified plant tissues are attacked on the surface of the soil or in its upper layers first of all by fungi, which thus become instigators of the decomposition of this comparatively resistant material. This primary attack which often transforms to a great extent the original substrate is naturally activated by the action of other components of soil microflora and proceeds towards further mineralization (Vladimir, 1970).

Dead organic matter constitutes one of the most

widely available sources of energy in the soil environment (Gray and Williams, 1971). Plant litter reaching the soil surface is immediately attacked by soil animals amd microbes so that new substrates are formed and substances released during the process pass down the soil profile. The importance of this fact has been realised and it has become more desirable to understand the problem after the researches of Patrick (1971), Patrick and Toussoun (1965), Patrick et al (1963, 1964) who reported the release of toxic substances during the decomposition of plant material. They further advocated that the nature and amount of toxic substances vary during decomposition with changed soil environment.

The fertility level and germination of seed sown in the field may be affected by toxic substances produced by decomposing plant litter. In some cases certain microbes associated with decomposing materials may also affect the sown seeds. The microfungal flora associated with decomposition process of the plant organs are highly influenced by environmental condition in which the plant is growing. Even in the case of fallen plant leaves, various degrees of decomposition viz; brown, blackish brown, decayed and heavily decayed are results of both the natural environment and fungal succession (Tubaki et al 1971).Various types of plant litter were recognized by Muller (1887) on the basis of their rates of incorporation into the soil. The slowly

decomposing litter forming a layer several centimeters thick over the soil surface, was termed 'mor' and is characteristic of coniferous forests. Examination of the litter in a pine forest by Kendrick and Burges (1962) exhibited several distinct layers viz.; L, F_1, F_2, H, each containing needles at different stages of decomposition. Kendrick and Burges (1962) have estimated that Pinus sylvestris needles spend about 6 months in the L layer, 2 years in the F_1 and 7 years in the F_2 before they become incorporated into the humus layer.However, during this process of decomposition of needles some soluble substances are released and leached into the soil by rain which may get into the soil quite quickly (Gray and Williams, 1971).

Succession on the leaves and constant patterns of decomposition of the organs by microfungi have been studied by Dickinson and Morgan-Jones (1966). Dickinson (1965) noted that three groups of phylloplane fungi are associated with Halimione leaves : those present only as propagules on the leaves, those growing actively and sporing on the leaves, and thirdly a group which only produce vegetative mycelium while the leaves remain green. At the onset of senescence, fungi in the latter group may sporulate and the leaf is also invaded by a number of saprophytes not actively associated with the phylloplane. However, not much has been known about the patterns of the first invaders

and their activities during the decay of the leaves either on the plant or on the litter surface for which the present investigation was undertaken.

The present investigation was aimed to study the following facts :

1) The effect of climatic fluctuations and age of the pine plantations on the occurence of litter decomposing microbes.

2) Pattern of fungal succession during pine litter decomposition.

3) Rate of pine litter decomposition under the stress of climate of this region.

4) To assess the microbial activity during litter decomposition by determining rate of substrate weight loss and by evolution of Co_2.

5) Types of phytotoxins released during decomposition process.

6) The role of some dominant and common fungi on pine litter decomposition under laboratory condition.

REVIEW OF LITERATURE

SUCCESSION :

Several researchers have worked out the succession of higher plants in different type of habitats. A number of workers have also highlightened the succession of microorganisms on various plant parts. Garrett (1951) inspired a number of workers to explore the various aspects of soil microbiology. Workers like Hudson (1968), Kendrick (1957, 1958), Mikola and Hintikka (1956), Pugh (1958), Saito (1956, 1960), Siu and Reese (1953), Smith and Wieringa (1953) Tribe (1957, 1960a, 1961), Tyner (1961), Waid (1957) and Webster (1956,1957) added further to the knowledge of succession of microorganisms on various plant parts.

Chester (1950) and Mangenot (1952) examined the succession of fungi on logs and wood and found different pattern of distribution of fungi. Hudson and Webster (1958) and Yadava (1966) investigated the succession of microbes on stem of different plants. Chang and Hudson (1967) in their studies on the fungal succession of wheat straw compost from ecological, biochemical and physiological point of view found a regular pattern of fungal colonization. Fungal succession on decomposing wheat straw compost in soil was examined by Sadasivan (1939). He noted a marked change in succession of fungi at different decomposition stages.

Most of the work on the microflora in relation to decomposition of coniferous leaf litter has been on pine litter

(Ward, 1952; Gremen, 1957; Tubaki and Saito, 1969; Brendsberg, 1969). These workers emphasized that the early colonizers of coniferous leaf litter are bacteria, ascomycetes, fungi imperfecti and some basidiomycetes which attack simple carbohydrates and cellulose. These organisms are followed by phycomycetes, particularly members of mucorales which can utilize the faunal breakdown products perhaps in combination with meio-fauna.

The most detailed study regarding the succession of fungi on decomposing coniferous litter is that of Kendrick (1958). He examined the litter in a stand of Pinus sylvestris at Delamare forest, England. His results seem to give the clearest idea available to date on succession. Waid (1952) in a detailed study of litter decomposition in pine woods, conducted near Nottingham, England listed about 120 fungi. The most frequently isolated genera were : Trichoderma, Penicillium, Mortierella, Mucor and Aureobasidium. Basidiomycetes were active in litter layer but decreased with increase in depth.

Garrett (1963) in his investigation of fungal colonization of dead plant remains in or upon the soil noted that sugar fungi were primary colonizers and compared the fungal succession with autogenic plant succession. Caldwell (1963) while studying the fungal flora of beech leaf litter in soil reported a definite

course of succession of the organisms. Carre (1964) confirmed the result of Caldwell and reported a definite succession in natural conditions. His results clearly demonstrated the effect of weather, which influenced the growth of microorganisms for first two years rather than by the accumulation and availability of nutrients.

Hayes (1965) in his studies of Scot pine litter decomposition reported that the microclimate influenced the development of dominant species of litter microflora Hering (1965) studied the fungal succession on four plant species and observed that the fungi were common in all four types of litter. The primary fungal flora was replaced by the common fungal flora dominated by the species of Penicillium and Trichoderma.

Macauley and Thrower (1966) observed succession of 5 groups of fungi during 15 months of decomposition of leaf litter of Eucalyptus regnans. Their report suggested that the primary colonizers were some moniliales followed by species of Penicillium and mucorales in advanced stage of decomposition. They suggested that probably antagonistic effect of other fungi was related to the disappearance of certain fungi even when sufficient nutrient was available.

Pugh (1958) suggested that the temperature and moisture content of leaf litter were the main factors to influence the seasonal activities of microbes during the decomposition of leaf litter. He also observed that the initial colonizers of dead and moribund leaves of

Carex paniculata were replaced by secondary colonizers during decomposition. Gregory et al (1963), Khanna (1964) and Sharma (1967) studied the microbial decomposition of above ground ageing and decaying plant parts and observed a climax succession at the senescence stage. Kamal and Singh (1970) and several others studied the fungal succession on foliage and stem and added further knowledge to the fungal succession.

Merril and French (1968) in their studies on decomposition of stalks of *Pinus ponderosa* isolated species of *Mucor*, *Rhizopus* and *Absidia* as dominant fungi. Rai (1969) reported several interesting species of saprophytic fungi during the studies of fungal succession on decaying leaves of *Saccharum munja*.

Frankland (1966,1969) investigated the succession of fungi on decaying petioles of *Pteridium aquilinum*. The aim of the experiment was to determine the relative abundance and succession of the various species of fungi in relation to changes in the substrates. In the experiment it was observed that a succession of species occured. Cellulose and lignin decomposers predominated before the appearance of sugar fungi. Seasonal variation of fungal population was also recorded.

Vladimir (1970) studied the chemical changes in lignocellulose decomposed successively by the fungi *Ganoderma applanatum* and *Serpula lacrymans* and observed

that the two fungi changes the degree of availability of lignocellulose for the other fungi, and these changes depend primarily on the changes of the polysaccharidic components.

Tubaki et al (1971) examined fungal succession on sterilized leaves of Castonopsis and Quercus in natural condition and observed a regular pattern of succession.

Rai (1973) while studying the fungal succession on decaying leaves of Saccherum munja noticed various kinds of fungi which appeared at various stages of decay and fungus flora was found to vary as the leaf tissues decomposed and disorganized. He recognized three groups of fungi for their distinct patterns of distribution. Watson et al (1974) studied in detail the fungal succession on loblolly pine litter and foliage in North Mississippi and could record a distinct successional pattern. Deuteromycetes, phycomycetes, ascomycetes and basidiomycetes were all prominent on F layer. Basidiomycetes seemed to be dominant in the H layer. An unique pattern of fungal succession on animal dung as substrate was described by Bell (1974). The seasonal cycles of the fungal flora on the dung was discussed. Some fungi were markedly seasonal in their occurence, others did not show such a marked seasonal pattern, but the fungus flora as a whole followed a predictable seasonal cycle with maximum fruiting of the species in winter and minimum in summer.

Vittal (1976) in his studies on fungal colonization of litter grouped the fungi as 'dominant' 'common','frequent', 'occassional',and rare'depending on their percentage frequency. He recorded only a few fungi as 'dominant' on each plant species and nearly half the number of species recorded occurred sporadically.

The studies of Pugh _et al_ (1975); Bashi _et al_ (1976, 1977) and Lindsey _et al_ (1976 a,b) showed the importance of yeast on leaf surface. Their role in litter decomposition needs thorough understanding of population dynamics and their successional pattern. The present investigation also shows a population dynamics of yeast flora on decomposing pine needles.

Mckenzie _et al_ (1976) during the studies of the decomposition of poplar leaves, plum leaves and wheat stems pointed out the importance of the primary fungal colonizers during decay after leaf fall. He observed that the rust infected plant materials supported a fungal succession quantitatively different from that on noninfected leaves and stems during decay.

Black _et al_ (1977) studied the pattern of colonization of scots pine litter by soil fungi and indicated that many common soil fungi, especially _Trichoderma_ spp., members of mucorales and _Penicillium_ sp, may colonize the surface, of decomposing needles in the litter but appear to have only a limited ability to colonize inner tissues.

Population dynamics of fungi, bacteria and actinomycetes in decomposing litter -

Much works have been done on fungal decomposition of plant remains on soil or inside soil by various workers. Works are also available on fungal colonization of different substrates under different environmental conditions. Mikola and Hintika (1956) found an overwhelming dominance of only a few species of bacteria in Pinus sylvestris litter in Finland. Inspite of the limitations of the experimental technique they drew some conclusion that sporulating moulds are abundant in forest soil. They play an essential role in the decomposition of litter mainly during early stages, whilst later on other fungi and bacteria attain more importance.

Witkamp (1963) made an annual means of fungal and bacterial counts from five leaf species decomposing in coniferous and deciduous stands and observed that fungal and bacterial counts were positively correlated with the corresponding rates of litter breakdown. He also observed that temperature, moisture and stage of litter decay, effectively controlled the microbial populations and rates of litter breakdown.

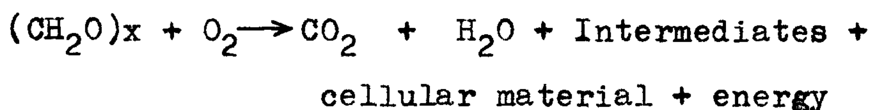
Witkamp (1966) also studied the bacterial and fungal population during decomposition of Mulberry, redbud, white oak, loblolly pine litter in Tennessee and found that microbial densities and annual weight losses of litter were significantly positively correlated.

Minderman and Daniels (1967) determined bacterial numbers in litter collected from a calcareous mull site in Holland at different times after leaf fall. In both cases maximum number was found a few weeks after shedding of the leaves, and this was followed by a gradual decrease. Similar results were obtained in a mixed Oak wood in Belgium (Remacle, 1970,1971), in stands of *Fagus sylvatica* in Germany (Meyer, 1960). In the latter case no consistent trends with regard to seasonal variation could be observed. Changing weather conditions affected the bacterial number more than the age of the litter. Few studies have been carried out on the composition of the bacterial flora in litter. Mangenot (1966) found that the bacterial flora on freshly fallen *Malus* leaf litter mainly consisted of yellow or orange pigmented forms as in the phylloplane. Holm and Jensen (1972) carried out a similar examination on *Fagus* litter, and they found a bacterial flora was very similar in composition to that on the mature leaves in the canopy.

Microbial activity and litter decomposition :

Studies of the decomposition of organic matter in forest soils are important for an understanding of soil fertility and the cycling of nutrients. Measurements of carbondioxide production in the field are frequently used for estimations of rates of organic matter breakdown, (Ross *et al* 1972).

Stotzkyy (1965) reviewed that when organic matter is attacked by microorganisms, the following reaction takes place :



$$(CH_2O)x + O_2 \rightarrow CO_2 + H_2O + \text{Intermediates} + \text{cellular material} + \text{energy}$$

Only 60-80 percent of the carbon is converted to carbondioxide, even under fully aerobic conditions because of incomplete oxidation of the substrate, which gives rise to intermediates, and the synthesis of cellular materials.

Mac Fadyen (1970) and others also studied the microbial activity by measuring rates of CO_2 evolution and litter decomposition.

Lamb (1976) studied the decomposition of organic matter on the forest floor of *Pinus radiata* plantations and measured the rate of litter decomposition by weight loss and by microbial respiration.

Bunnell *et al* (1977) used simulation models to examine the role of substrate chemistry in decomposition rates by relating specific laboratory measures of microbial respiration to field measure of weight loss with those influences of temperature and moisture.

Carbondioxide evolution has been used frequently as a measure of biological activity in the litter (Miller, 1974). Kornev (1962),working on *Pine* found that the evolution of CO_2 decreased in the litter and suggested that this decrease was related to the unilateral consumption by microorganisms of the available organic matter in the upper layers, with consequent accumulation of unavailable compounds. The evolution of CO_2 varied with the season, increasing

through the summer to a maximum in August with a second peak in October following needle fall.

Phytotoxins :

There are evidences that decomposition of plant residue in the soil results in the formation of compounds which may have either favourable or unfavourable effects on plants. The speculations raised at the beginning of the century with respect to phytotoxic properties of some of these compounds, have now been confirmed.

The beneficial or detrimental effects of certain substances released by various plant parts in soil during decomposition were first reported by De Candolle (1832). Skinner (1918) studied the relative toxicity of these compounds in soil and nutrient solutions. He identified several compounds released during decomposition of plant residues in soil and referred them to be the degradation products of lignin. Mc Cella and Duley (1950) and Guenzi and Mc Calla (1962) studied the inhibitory substances produced in soil during wheat straw decomposition and noted 66 percent inhibition of corn seed against only 10 percent in control. Further knowledge regarding the production of phytotoxins during decomposition of plant remains in the soil was added by the works of Proebsting (1950), Martin (1957), Patrick and Koch (1958), Snyder et al (1959), Nielson et al (1960) and Sondheimer (1961).

Nielson et al (1960) reported the abnormal behaviour of phytotoxins on many seeds when they are soaked in the extract of decomposed straw. The presence

of Ferulic acid, Coumaric,acid, Vanillic acid and P-hydroxybenzoic acid was reported by Borner (1960) from aquous and alcoholic extracts of straw and roots of barley, rye and wheat. Patrick (1955) and Patrick and Koch (1958) studied the production of phytotoxins by decomposing plant remains in soil.

Patrick et al (1964) presented a comprehensive review on the products of crop residue decomposition and their effect on plant roots. Toussoun et al (1968) and Patrick (1971) studied the nature of phytotoxic substances produced during plant decomposition in soil. They explored this aspect of soil microbiology and confirmed the findings of previous workers.

In India, studies on this aspect of soil microbiology have been very few so far. Kanaujia (1973) investigated the production of phytotoxins during the decomposition of roots of Pennisetum typhoides (Burm F.) in field conditions. He detected several phytotoxic substances during various stages of root decomposition. Recently Grover (1977) also studied the production of various types of phytotoxins from root,leaf and stem of Brassica nigra var. Sarson during decomposition in soil. He has also studied the effect of these decomposition products on the seeds of various crop and also on the important fungi of soil.

MATERIALS AND METHODS

A. Field decomposition studies :

The present investigation was carried out under 1955, 1965 and 1970 Pine plantations as shown in the Map 2 where the investigation on leaf surface microflora and soil microbial population was also conducted. Different age groups of pine plantations were considered to study the effects of age of the pine needle and plantations on litter decomposition.

In this investigation following parameters were considered :-

1) Rate of pine litter decomposition on the forest floor.

2) Population dynamics of litter microflora (bacteria, fungi, actinomycetes) and the pattern of fungal succession during the litter decomposition. pH and moisture content of the litter and different climatic conditions of the sites were also taken into consideration.

3) Evolution of Co_2 from litter during decomposition.

4) Degradation of carbohydrates, cellulose, hemicellulose, lignin and release of aminoacids of litter during decomposition.

5) Release of phytotoxins during decomposition process.

1) Rate of pine litter decomposition on the forest floor :-

Nylon bag technique (Bocock, et al 1960) was applied to study the rate of litter decomposition of *Pinus kesiya* on the forest floor. Nylon nets having 0.5 mm mesh were

stitched into 12 sq.cm. bags. Fresh pine needles were collected from 1955, 1965 and 1970 plantations and air-dried till the constant weight was recorded. The bags for 1955 pine plantations were filled with 8.366 gms. of air-dried pine needles. Likewise the bags for 1965 and 1970 plantations were filled with 8.466 gms. and 8.600 gms. of air dried pine needles respectively. All the bags were then properly stitched and placed on the forest floor of 1955, 1965 and 1970 plantations on 15th.May, 1976. The litter in the bags was then allowed to decompose. The first sampling was done on 15th.June, 1976.and the subsequent samplings were done at one month interval for a period of one year. On each sampling date six replicate bags were collected from each plantation for recording the loss in dry weight and microbial population of the litter. The bags were brought to the laboratory. Three bags were made open and the other three bags from each set were kept in freeze until used for microbial analysis. From each bag, litter was carefully seperated, cleaned from any adhering soil particles and was allowed to dry in a hot air oven at a temperature of 80°C until a constant dry weight was obtained. Final dry weight of the samples was taken and percentage weight loss was calculated on the basis of oven dry weight of the sample.

The experiment was repeated for two successive years from May 1976 - May 1977 and May 1977 - July 1978 to observe the variation in litter decomposition. Only two pine plantations viz. 1955 and 1970, were considered during

the second year for the study of litter decomposition. The bags containing 4.850 gms. and 4.800 gms. of air dried fresh pine needles for 1955 and 1970 plantations respectively were kept on the forest floor of both the plantations on 14th.May 1977 and allowed to decompose as described earlier. The collection of the bags and estimation of dry weight loss of litter was carried out as described earlier. The first sampling during second year was done on 14th.June, 1977 and subsequent samplings were done at one month interval for a period of one year.

The course of changes in pH and moisture content of decomposing litter were also recorded for two years.

2) Population dynamics of litter microflora (bacteria, fungi, actinomycetes) and the pattern of fungal succession during pine litter decomposition :-

The aim of this experiment was to evaluate the saprophytic activity of microflora during pine litter decomposition on the forest floor, and also to assess the pattern of fungal succession during the decomposition. The experiment was also repeated for two years i.e. from May 76 - May 77 and May 77 - May 78.

Collection of the samples - Nylon bags containing decomposing litter were collected from the different plantations, The bags were properly sealed and transported to the laboratory and were used on the same day for the assessment of microflora. Occasionally the bags were stored overnight at 4°C when the processing could not be completed the same day (Ruscoe 1971). Additional litter bags were collected for the determination of pH and moisture content.

<u>Isolation of microflora</u> :- The process consisted of the following steps :

<u>Isolation of bacteria and Actinomycetes</u> - Dilution plate method was followed for the isolation of bacteria and actinomycetes throughout the sampling period. One g of decomposing pine litter was introduced into a 250 ml. sterilized conical flask containing 100 ml sterilized distilled water. Litter suspension of 1:100 dilution was prepared. The flask was thoroughly hand shaken for fifteen minutes for homozenizing the suspension. 10 ml of this suspension was then transferred aseptically by means of a sterilized 10 ml Pipette to another 250 ml sterilized conical flask containing 100 ml sterilized distilled water to get a suspension of 1:1000 dilution. The process was repeated once more to get a suspension of 1:10000 dilution which was found suitable for counting bacteria and actinomycetes colonies on the agar plate. Isolation of bacteria was done on nutrient agar medium (Difco manual, 1953) containing beef extract. Isolation of actinomycetes was done on starch casein agar plus antibiotics medium which has been shown to be highly selective for the microorganisms (Kuster and Williams, 1964). The selectivity of this medium was further increased by the addition of actidione and nystatin, each at a concentration of 50 Mg/ml of medium (Williams and Davies, 1965). Composition of both the media is described in Chapter 1 under materials and methods.

0.5 ml of litter suspension in each plate of 1:10000 dilution was transferred aseptically on the

nutrient agar medium and starch casein medium in sterilized petriplates for the isolation of bacteria and actinomycetes respectively. Isolation was carried out in sterilized Lamminer flow chamber throughout the investigation period. Three replicate plates were maintained for each case. The petriplates were shaken gently to disperse the litter suspension uniformly over the surface of the agar medium. The plates were incubated upside down at a temp. of 30 ± 1°C in a bacteriological incubator. The plates for bacterial and actinomycetes counts were incubated for 24 hours and 7 days respectively.Number of bacteria and actinomycetes colonies was counted with the help of a digital colony counter. The population of bacteria and actinomycetes/g dry litter was calculated by taking moisture content of the litter into account. In this study only population dynamics of bacterial and actinomycetes was considered and no attempt has been made to identify the species.

Isolation of mycoflora - Cultural isolation technique (dilution plate method) was employed for this group as described in the Chapter 2.The results were represented in terms of percentage frequency and percentage relative abundance of fungi as described in Chapter 1.

pH and moisture content of litter - pH and moisture content of the decomposing litter samples during different sampling periods were determined by oven dry method and electric pH meter respectively.

Climatic conditions - The data on atmospheric

temperature, rainfall and humidity was obtained from the local materiological office throughout the investigations.

3) <u>Evolution of Co_2 from litter during decomposition:-</u>

Determination of Co_2 evolution from decomposing litter indicates the rate of breakdown of litter on the forest floor. Only one plantation was considered for this experiment.The experiment was conducted for a period of one year fram May 1977 to May 1978.

Co_2 evolution from decomposing Pine litter from the forest floor was estimated by inverted box method (Walter and Haber, 1957).Glass beakers of 100 ml capacity containing 50 ml of O.IN KOH solution were exposed on the surface of decomposing litter on the forest floor of 1970 plantation. The whole system was then covered by means of tin containers (5071.2 cms. sq. area) in such a way that there was no leakage from the sides of the containers. The assembly was kept on the forest floor for 24 hours to allow KOH to be saturated with Co_2. After 24 hours of exposure of the absorbent solution (KOH) the amount of Co_2 fixed by the KOH solution was measured by titrimetric method using 0.1 N Hcl and phenolphthelin as indicator. Three replications were used in each case.Finally Co_2 evolution was expressed in $mg/m^2/hr$.

Control sets were also kept side by side. A small area of the forest floor (according to the diameter of tin used) was covered by means of a polythene sheet to ensure no Co_2 accumulation take place from the forest floor and the whole system along with KOH solution was covered by

LIBRARY

inverted tin. The other processes followed were similar as done for the samples.

4) Estimation of cellulose, hemicellulose and lignin of the decomposing pine litter :-

Cellulose, hemicellulose and lignin content of Pine litter was estimated by method described by Peach and Tracey (1955). Decomposing leaf litters collected from litter bags on different sampling dates were washed with distilled water to remove all the soil particles, and dried in a hot air oven at 80°C for 48 hours. The samples were cooled to room temperature over anhydrous calcium chloride in a desiccator and were ground to find powder by electric grinder and processed for estimation of the above 3 compounds.

0.5 g of powder from each plantation (for each sampling date) was treated with 25.0 percent of aqueous KOH (W/V). In each case, the samples were centrifuged at 3000 rpm for 10 minutes. The decant obtained in each case was used for detection of hemicellulose. The residue, left at the end of the digestion, was washed several times in distilled water till the trace of KOH was removed. It was dried in a hot air oven at 105°C for 24 hours, cooled to room temperature in a desiccator and weighed. The amount obtained was designated as total cellulose. The decant in each case was collected and neutralized with equal amount of glacial acetic acid and ethanol. The precipitate was filtered washed, dried and weighed as above and this accounted for total hemicellulose. Three replicate samples

were considered in each case.

For estimation of lignin 0.5 g dried samples, were taken in a test tube seperately for each set, treated with 72 percent sulphuric acid (AR) and kept in a deep freeze for 24 hours. After 24 hours the content of the test tube was centrifuged and decanted. The clear solution was discarded whereas the residue was washed many times till no trace of H_2So_4 was present. It was finally washed, dried and weighed. The amount so obtained was designated as Lignin. Three replicate samples were considered in each case.

The cellulose, hemicellulose and lignin contents were estimated on the initial dry weight of the decomposing litter. Fresh litter samples were also analysed side by side.

5) Estimation of total sugar and total aminoacid from the decomposing litter samples :-

Estimation of total sugar and total amino acid of the decomposing litter was carried out as described by Peach and Tracy (1955). 100 mg. of powdered sample from each sample and from each plantations was crushed properly in a morter and pastle in 80% ethanol and filtered through a Whatman filter paper No.1. A slightly yellow coloured filtrate was obtained in each case. The filtrate was treated with actimated charcoal and centrifuged at 6000 rpm to get a clear supernatant. Ethanol was boiled off from the clear filtrate in a hot water bath and the volume was made up to 5 ml with the addition of double glass distilled water. 3 ml of this solution was

taken in a test tube and 6 ml of Anthrone reagent (0.4% in H_2So_4 w/v) was added gently to the test tube from the side while the test tube was kept in a cold water bath. Then the test tube was gently shaken and warmed in a boiled water bath for 3 minutes. A green colour was obtained. The transmittance of this coloured solution was observed in spectrophotometer (Spectronic 20) at 610 mμ. Standard curve was obtained from transmittance of varying conc.of glucose solution treated with anthrone reagent as described for the samples. From the standard curve the values of the total sugar of the samples were expressed in μg/100 mg dry weight of the samples.

To the rest of the 2 ml extract, 2.5 ml of acetate buffer (40 g NaoH dissolved in distilled water in a 1000 ml volumetric flask, 100 ml of glacial acetic acid was added to it and the volume was made upto 1000 ml with distilled water) and 2.5 ml of ninhydrin solution (1% in isopropyl alcohol) were added simultaneously.The test tube containing the solution was placed in hot water bath for half an hour, a light purple colour was obtained. The transmittance of this coloured solution was observed in spectrophotometer (Spectronic 20) at 540 nm.

Standard curve was obtained from the transmittance of the varying concentration of leucine solution treated with acetate buffer and ninhydrin solution as described for the samples. From the standard curve the values of the total amino acid of the samples were expressed in μg/100 mg dry weight of the sample.

The estimation in each case was done in triplicates.

6) Collection of litter leachates and detection of phytotoxins :-

During each sampling one litter bag from each plantation was kept separate for collection of litter leachates from the decomposing material. Nylon bags with decomposing pine litter and adhering soil particles were immediately transferred to 250 ml conical flasks having 100 ml. sterilized distilled water. Each flask was hand shaken vigorously for 20 minutes keeping the mouth closed with sterilized cotton plugs. This washing was then filtered through ordinary filter paper and the filtrate was centrifuged at 3000 rpm for 10 minutes. The supernatant was again filtered through a Whatman filter paper No.1. This filtrate was then concentrated in a vacuum oven below 40°C upto 5 ml and was kept in a laboratory freeze until used for the detection of phytotoxins present in the leachates.

Phytotoxins from the decomposing litter leachates were detected by unidirectional paper chromatography using isopropanol, ammonia and water (200:10:20) as solvent (Smith, 1960) and ferric chlorode solution ($Fecl_3$ 2% in water containing 1 ml of 2N Hcl) as location reagent (Hathway,1960).The spots on the chromatograms were examined properly under ultraviolet ray in a dark room for identification of phytotoxins. The unknown spots appeared on chromatograms were compared with those of standard phytotoxins. The Rf values of the unknown spots

were calculated and compared with the Rf values of standard phytotoxins. Eight standard phytotoxins used for this purpose were 3,4 dihydroxybenzoic acid, P - coumaric acid, ferulic acid, venillic acid, quinic acid, protocatechuic acid, gentisik acid and caffeic acid.

B. Decomposition studies in laboratory :

The experiment was conducted with a view to measure the rate of pine litter decomposition by certain selected fungal species and to compare laboratory decomposition vs. field decomposition studies.

Six dominant and common soil and litter fungi viz., Absidia cylindrospora, Mucor hiemalis, Trichoderma viride, Penicillium chrysogenum, Cladosporium herbarum and Sporobolomyces roseus were selected for this study.

1) Inoculation of sterilized soil and sterilized pine litter :-

Rectangular glass jars (12.5 cm. x 10.3 cm. x 20.8 cm) were used for this purpose. Soil from 1955 pine plantation of the experimental site was brought into the laboratory and was properly sieved to remove the adhering plant parts and debris. Oat meal was added @ ten g per Kg. of soil to support the fungal growth in the soil after inoculation. Soil was properly mixed with Oat in alluminium trays and was autoclaved at 15 lb/sq in. pressure for 3 hours. Autoclaved soil was then allowed to cool down to room temperature in an aseptic condition.

Pure cultures of all the selected fungi were maintained in petridishes on malt extract. With the help of a cork borer (1cmdia) 10 pieces, seven days old culture

of each fungus was introduced in sterilized soil. The pieces were then thoroughly mixed in soil in an aseptic conditions. For each fungal culture separate sterilized soil was used. Sterilized rectangular glass jars were then filled with inoculated soil @ one kg./jar, and was levelled properly for different fungal inoculum. Mixed inoculation of fungal cultures in autoclaved soil was also done. Six replicate sets were maintained for each inoculum including uninoculated control set for each sampling periods. After inoculation all the jars were covered with polythene sheets having minute pores made by a needle to avoid air contamination. The glass jars were then incubated at 25°C in BOD incubators for one month for proper growth and establishment of inoculated fungi in soil.

Moisture content of the soil was maintained at field capacity throughout the experiment by adding requisite amount of sterilized distilled water after every week.

After one month of incubation, fungi were established properly in soil and their growth was prominent in soil, except that of Sporobolomyces roseus.

Pine needles for this study were collected from the oldest i.e. 1955 plantation from the experimental field and were air dried properly.

Nylon bag technique (Bocock, 1960) was applied as described earlier for laboratory decomposition studies. Nylon nets having 0.5 mm mesh size were stitched into 12 sq.cm. bags which were then filled with 8.359 g. (dry wt. basis) of fresh pine needles. Bags were properly stitched.

After one month when the fungal cultures were properly, established in the soil, one litter bag was introduced in each jar on 5th.April,1978. The litter bags were placed in all the jars on the same day. Before introducing the bags in the jars the needles were autoclaved at 10 lb/sq.inch Pressure for 15 minutes (Black and Dix, 1977).The jars were then placed at room temperature for incubation. The moisture content of the litter was maintained at 35% throughout the course of investigation by adding requisite quantity of sterilized distilled water from time to time. The growth of the fungus on litter surface in each jar was examined thoroughly from time to time.

2) Weight loss study :-

The first sampling was done after one month of the incorporation of litter bags in the jars on 6th.May, 1978 and the subsequent samplings were done on the same date of June, July, September, November upto January 1979 for a period of nine months.

Three jars were removed from inoculated sets and three from uninoculated sets respectively on each collection date.The jars were opened and bags were collected for the determination of weight loss. The bags were opened in the laboratory and the litter was carefully separated and cleaned with the help of forcep to remove all the adhering soil particles and then dried in a hot air oven in petridishes at 80°C until a constant dry weight was obtained. Final dry weight of the samples was taken in electric

balance and the percentage loss was calculated on the basis of oven dry weight of litter.

3) Chemical analysis of decomposing litter samples :-

Cellulose, hemicellulose and Lignin content of decomposing litter samples of all the sets was estimated as described earlier in the methods of field decomposition studies (Chapter 3).

Collection of litter leachates and detection of phytotoxins were also carried out by the methods described earlier in the chapter 3.

4) Estimation of Co_2 evolution from decomposing pine litter:-

Co_2 evolution from litter was estimated from inoculated and uninoculated sets respectively on each sampling date, before the bags were taken out for the determination of weight loss.

To measure Co_2 evolution 20 ml of 0.1 N Sodium hydroxide solution plus phenolphthelin indicator were pipetted into 50 ml beakers, these were then placed inside the rectangular glass jars containing litter bags. The glass jars were then sealed with airtight lids. After the appropriate exposure time (24 hours) the jars were opened and excess barium chloride (10% of the volume of absorbent used) was added to the absorbent to precipitate Potassium carbonate (Macfadyen,1970). The beakers were then taken to laboratory and titration of the residual NaoH was made against 0.1 N hydrochloric acid. The volume of NaoH reacting with Co_2 was measured following Macfadyen (1970), 1 ml of 0.1 N NaoH used was taken to represent the absorption of

1.118 ml of Co_2. During the course of each exposure period the minimum and maximum soil temperature was recorded from the jar and a mean temperature was calculated.

The measurement of Co_2 evolution from soil without litter bag was also done for control and finally the reading of Co_2 evolved from litter was substracted from the reading of Co_2 evolved from soil.

The result of Co_2 evolved from litter was then expressed in ml/hr.

RESULTS

The experimental findings on litter decomposition are represented in the following order :

A. Field decomposition studies :

1. Quantitative assessment of fungi, bacteria and actinomycetes of pine leaf litter at different periods of decomposition during 1976-1977 and 1977-1978 are represented in the Figs.21-24. Statistical analyses of the results are depicted in the tables 60-65.

2. Records of percentage relative abundance and frequency of fungal species from pine litter during different periods of decomposition are represented in the tables 66-69 and Figs.25-28.

3. Rate of pine litter decomposition on soil under different plantations during 1976-1977 and 1977-1978, also the course of change in pH and moisture content of litter during decomposition are represented in the figs. 29-31. Statistical analyses of the results are depicted in the tables 70-75.

4. Records of Co_2 evolution from decomposing pine litter under natural condition and its possible correlation with moisture content and total number of microbes are depicted in the fig.32.

5. Quantitative estimation of cellulose, hemicellulose, lignin, total soluble sugar and total aminoacids present in pine leaf litter in different periods of decomposition during 1976-1977 and 1977=1978 are represented in the figs. 33-36.

6. Types of phytotoxins released from litter during decomposition under natural condition are depicted

in the figs. 40-44.

B. Laboratory decomposition studies :

7. Morphological features of pine litter.

8. Dry weight loss and chemical changes in decomposing pine litter by various fungal species are represented in figs. 37-38, and their statistical analyses are represented in the tables 76-79 and 81.

9. Changes in pH of pine litter at various periods of decomposition is represented in the table 82.

10. Rate of Co_2 evolution from decomposing litter by different fungal species are represented in the fig.39 and their statistical analyses are depicted in the tables 80 and 81.

11. Release of phytotoxins during different periods of decomposition by different fungal species are represented in the figs. 45-47.

A. Field decomposition studies :

(1) Quantitative assessment of fungi, bacteria and actinomycetes on pine litter in different periods of decomposition under natural condition :-

The fungal population of decomposing pine litter was minimum on first sampling date and the maximum at the end of the decomposition. This trend was almost similar in all the three plantations viz. 1955, 1965 and 1970, studied during 1976-1977 (Fig.21). The population gradually increased when the decomposition of the litter progressed. During the first few months of decomposition the increase in fungal population was rapid when the moisture content

was also quite high (Fig.21). The fungal population on Pine litter of both 1955 and 1965 plantations attained its peak in the month of September when the moisture content was very high (Fig.21). The population, thereafter rapidly decreased. The moisture content of decomposing litter at this stage also declined. The least population was observed in the month of January when the moisture content of the litter was below ten percent (Fig.21). Thereafter, the fungal population of the decomposing litter increased again and reached at its peak in the month of May (Fig.21). The change in fungal population of litter of 1965 plantation exhibited a different tendency (Fig.21). Statistically significant positive correlation was observed between the fungal population and moisture content of decomposing pine litter of all the plantations as the values for the simultaneous measurements of moisture content and fungal population was found to be highly correlated ($r = 0.738$, $P < .01$ for 1955 plantation and $r = 0.501$, $P < .10$ for 1965 plantation respectively). Statistically no significant variation in the fungal population of decomposing pine litter was observed between the plantation studied during 1976-1977 but a significant variation was observed between the different periods of decomposition (Table 60).

During the second year of investigation, the fungal population of decomposing leaf litter of 1970 plantation exhibited a different picture. Though the population was minimum in the beginning of decomposition, it gradually increased and attained a peak in the month of

July (Fig.24). No direct correlation could, however, be observed between the fungal population and moisture content. Different pattern was observed in the change of fungal population of decomposing litter in the second year (Fig.24). Statistically no significant variation was observed in the fungal population of decomposing pine litter of 1970 plantation between first and second year studies (Table 63).

The bacterial and actinomycetes population of pine litter was also less in the first sampling of decomposing litter during 1976-1977 and increased significantly at the end of the decomposition studies. The population of bacteria and actinomycetes seems to be regulated by the moisture content (Figs. 22,23). The same trend was observed for all the three plantations. The population of bacteria and actinomycetes on decomposing pine litter in all the plantations increased rapidly as the decomposition progressed. The population of bacteria attained its peak in the month of July, whereas the population of actinomycetes was maximum in the month of September in all the plantations when the moisture content was more than 60 percent. The population thereafter declined and so was the case with moisture content (Fig.22) This decreasing trend in the bacterial and actinomycetes population on decomposing litter was observed upto February after that the population increased again and this trend continued upto the end of the decomposition

studies (Figs. 22,23). Statistically a significant positive correlation was observed between bacterial population and moisture content of decomposing litter of all the plantations ($r = 0.884$, $P < .01$ for 1955 plantation, $r = 0.600$, $P < .05$ for 1965 plantation and $r = 0.654$, $P < .05$ for 1970 plantation). Statistically a significant positive correlation was also observed between actinomycetes population and moisture content of decomposing litter of all the plantations ($r = 0.521$, $P < .10$ for 1955 plantation, $r = 0.648$, $P < .01$ for 1965 plantation and $r = 0.693$, $P < .01$ for 1970 plantation respectively.

Statistically no significant variation in the bacterial population of decomposing litter was observed between different plantations but a significant variation was observed between the different periods of litter decomposition (Table 61). Variation in the actinomycetes population of decomposing litter between different plantations and between different periods of decomposition was statistically significant (Table 62).

An interesting phenomenon was noticed for the growth of fungi, bacteria and actinomycetes on decomposing pine litter during 1976-1977. As soon as the moisture content of decomposing litter crossed more than 70 percent the population of all the three groups declined very rapidly and the population could not increase beyond this range of moisture content (Figs. 21,22,23).

Same trend in the change of bacterial and

actinomycetes population of decomposing litter of 1970 plantation was observed in the second year (Fig.24). Both bacterial and actinomycetes population increased gradually as the decomposition proceeded and attained a peak in the month of September (Fig.24) and population decreased thereafter when moisture content of litter crossed more than 70 percent. Statistically no correlation was observed between bacterial population and moisture content of decomposing litter in second year but a significant positive correlation was observed between actinomycetes population and moisture content of decomposing litter ($r = 0.749$, $P < .10$).

Statistically no significant variation was observed in the population of bacteria and actinomycetes on decomposing pine litter of 1970 plantation between first and second year studies (Tables 64 and 65).

2. <u>Records of percentage relative abundance and frequency of fungal species isolated from decomposing litter (based on dilution plate method)</u> :-

Fourteen fungal species viz. 4 phycomycetes, one ascomycetes (unidentified), 5 members of fungi imperfecti, 3 sterile mycelia and one member of yeast were isolated from the decomposing pine leaf litter from litter bags of 1955 plantation (Table 66 and Fig.25). *Trichoderma viride* was dominant fungus according to its percentage frequency (Table 66 and Fig.25). One sterile white mycelia was very common on decomposing litter and the rest fungi occured sporadically. *Penicillium chrysogenum*, *Trichoderma viride*, *Cladosporium herbarum*, *Aureobasidium pullulans* and a

sterile white mycelia were isolated as initial mycoflora of fresh pine needles before the litter bags were kept on the forest floor (Table 66). At the early stage of decomposition two members of phycomycetes like *Pythium* sp. and *Mucor hiemalis* were observed to be dominant fungi in the months of July and August respectively (Fig.25), but one unidentified ascomycetes out-numbered the phycomycetes in the month of September. Thereafter, members of fungi imperfecti were observed to be the dominant fungi of decomposing litter upto the end of the studies (Table 66 and Fig.25).The relative abundance of *Aureobasidium pullulans* was very low in decomposing litter.

Thirteen fungal species, viz; 3 phycomycetes, seven members of fungi imperfecti, one sterile white mycelia and two members of yeast were isolated from the decomposing pine litter of 1965 plantation. Not much variation was observed in the quality of mycoflora present on the decomposing litter of 1965 and 1955 plantation. *Penicillium chrysogenum*, *Trichoderma viride*, and sterile white mycelia were very common fungi on the decomposing litter of 1965 plantation (Table 67). *Penicillium chrysogenum*,*Trichoderma viride* and *Cladosporium herbarum* were isolated with very high relative abundance in some months from decomposing litter (Fig.26). Though members of phycomycetes like *Mucor hiemalis*, *Pythium* sp. and *Absidia cylindrospora* took part in the decomposition during different periods yet their frequency and relative abundance were very low in comparison with other fungi. It was observed from this plantation that the different members of fungi imperfecti

were dominant on the pine litter from early to later stages of decomposition (Table 67 and fig.26). Aureobasidium pullulans and Sporobolomyces roseus were isolated from the decomposing litter but their percentage relative abundance and frequency were very low.

Fourteen fungal species viz. 3 phycomycetes, seven members of fungi imperfecti, three sterile mycelia and one member of yeast were isolated from the decomposing pine litter of 1970 plantation. Qualitatively almost similar forms of fungi were isolated from this plantation as in case of the other two plantations. Penicillium chrysogenum was a common fungus of the decomposing litter of this plantation (Table 68, fig. 27). At the early part of decomposition two members of phycomycetes, Pythium sp. and Mucor hiemalis were dominant on decomposing litter. Later on different members of fungi imperfecti were dominant on the decomposing litter (Fig.27).

During the second year investigation of pine litter decomposition almost a different picture of mycoflora was obtained. Only nine fungal species viz. 2 phycomycetes, five members of fungi imperfecti, one sterile mycelia and a yeast species were isolated from the decomposing pine litter of 1970 plantation. Penicillium chrysogenum was dominant (Table 69 and Fig.28).

It was observed from this investigation that two members of fungi imperfecti viz., Penicillium chrysogenum and Fusarium sporotrichoides were dominant forms on pine litter at its early stage of decomposition from June - September. As the decomposition was proceeding further

these two fungi were outnumbered by Sporobolomyces roseus with very high percentage relative abundance upto the end of the studies (Fig.28).

3. Rate of pine litter decomposition on soil under different plantations and course of change in pH and moisture content of litter during decomposition :-

The rate of leaf litter breakdown on pine forest soil during 1976-1977 was comparatively slow than that of second year studies i.e. during 1977-1978, (Figs. 29 and 30). During the first year of observation it was found that weight loss of Pine litter was proceeding at extremely slow rate in all the three plantations. Not more than 20 percent of litter could decompose on the soil of 1955 and 1970 plantation even after six months of exposure to the soil (Fig.29), but almost 30 percent was decomposed in 1965 plantation. Thereafter pine litter, lost weight at a fairly constant rate over most of the study period (Fig.29). After one year only 30 percent of the litter was decomposed. Statistically, a significant variation in litter decomposition was obtained between plantations and between different periods of decomposition during 1976-1977 (Table 70).

In the second year it was observed that rate of disappearance of pine litter was little faster on the soil of both 1955 and 1970 plantation, and more than 20 percent of litter was decomposed after six months from the onset of decomposition studies (Fig.30). However, more than 30 percent of litter was decomposed after one year in 1955

plantation and more than 35 percent in 1970 plantation. Statistically no significant variation was observed in the decomposition of pine litter between different plantations and between different periods of litter decomposition (Table 71) during 1977-1978.

Moisture content and pH of decomposing pine litter varied much during different periods of decomposition in all the plantation during 1976-1977. At the very early part of decomposition i.e. in the months of June, July and August the moisture content of decomposing litter was extremely high. During the periods moisture content reached above 70 percent in all the plantations (Fig.31). Thereafter, the value decreased and was below 20 percent in the month of February and again it increased upto the month of May. At the onset of decomposition the pH of the litter was above 5 in all the cases. As the decomposition proceeded further litter exhibited low pH value and during the month of December and January the litter became highly acidic (Fig.31). Statistically a significant positive correlation was obtained between moisture content and pH of decomposing litter of 1955 and 1970 plantation ($r = 0.600$ $P < .05$ for 1955 plantation and $r = 0.792$, $P < .01$ for 1970 plantation respectively). Such a correlation was not obtained for the litter of 1965 plantation.

Statistically no significant variation in the moisture content and pH of decomposing litter was observed between different plantations but significant variation was obtained between different periods of decomposition

(Tables 72,73). Statistically no significant variation was obtained in the moisture content of decomposing pine litter of 1970 plantation between 1976-1977 and 1977-1978 observations, but there was a significant variation in the pH (Tables 74 and 75).

4. Evolution of Carbon dioxide from decomposing pine litter and its possible correlation with moisture content total microbial population and rate of decomposition :-

Statistically no strong correlation was observed between Co_2 evolution and moisture content of decomposing pine litter of 1970 plantation during 1977-1978. With an increase in the moisture content of decomposing litter a corresponding increase in the Co_2 production was observed upto the month of September 1977 (Fig.32). Thereafter, however, it showed an indistinct tendency. Highest amount of Co_2 evolution from litter was observed in the month of September when moisture content was very high (Fig.32). However, values for simultaneous measurements of Co_2 production and rate of litter decomposition on the forest floor on the otherhand was found to be positively correlated ($r = 0.769$, $P < .10$).

From this experiment a strong positive correlation was also observed between Co_2 production and total microbial population of decomposing litter (Fig.32) as the values for Co_2 production and total microbial population were highly correlated ($r = 0.810$ $P < .001$).

5. <u>Estimation of nonsoluble components like cellulose, hemicellulose, lignin and soluble components like total soluble sugar and total aminoacid present in pine litter during different periods of decomposition</u> :-

The initial amount of cellulose and hemicellulose fractions were almost similar in the litter of 1955,1965 and 1970 plantations (Fig.33).The change in amounts of these two components was almost similar in the litter of all the three plantations during 1976-1977. The loss of both the constituents followed total weight loss of pine litter very closely. Both the cellulose and hemicellulose fractions decreased more slowly during the first 3 months, before declining rapidly in the later stages of decomposition. However, percentages of hemicellulose remained relatively constant in the leaf litter of all the three plantations throughout the study period (Fig.33). The rate of cellulose breakdown was much faster than the rate of hemicellulose and almost 50% of cellulose was lost from the pine litter in one year period. This was similar for all the three plantations (Fig.33). during 1976-1977. Like hemicellulose and cellulose, the initial content of the lignin was also almost same in the pine litter of 1955, 1965 and 1970 plantations (Fig.33). The rate of disappearance of this component was much lower than that of the cellulose component. In fact a constant amount was observed for this fraction after two months of exposure of litter on the forest floor upto January before declining rapidly at the later stages of decomposition (Fig.33). The

observation was similar for all the three plantations, during 1976-1977. Almost a similar result was obtained for these three components during second year of investigation for litter decomposition in 1977-1978 (Fig.34). The initial contents of cellulose, hemicellulose and lignin components were same in the litter of both 1955 (oldest) and 1970 plantations. The loss of cellulose was more slower during the first four to five months before declining rapidly at the later stage of decomposition (Fig.34) but percentage of hemicellulose remained relatively constant throughout the study period. The rate of disappearance of lignin content in decomposing litter exhibited a similar trend like the first year observation.Almost a constant amount of this component was observed in the decomposing litter upto January (Fig.34).

Changes in concentration of total sugar and total amino acid, considered to be two major soluble components from pine needles, were followed throughout the study period, during 1976-1978 (Figs. 35 and 36). The initial concentration of total sugar was higher in the pine needles of 1965 and 1970 plantations than in the pine needles of 1955 plantation during 1976-1977 observation (Fig.35). However, the initial concentration of total amino acid was almost same in the pine needles of all the three plantations during 1976-1977 (Fig.36). Both these components decreased very rapidly during the first one month of litter decomposition (Fig.35 A and Fig.36 A).

However, after one month of litter decomposition an absolute increase in the concentration of total sugar in pine litter of 1970 plantation was measured and this increased in concentration continued for the first four months, before declining rapidly in the later stages of decomposition (Fig.35 A). In fact an increase in the concentration of total sugar was also noticed though less in pine litter of other two plantations during the early stage of decomposition but later on concentration decreased rapidly. The least amount of total sugar was measured in the last observation of the experiment for all the plantations (Fig.35 A).

Same was the case with total amino acid whose concentration also increased during the early stage of decomposition. After four months of decomposition, however, a rapid decrease was marked in the case of all the three plantations and it continued till the end of study (36 A). The least concentration of amino acid was noticed in the last observation.

A different picture was, however, noticed in the change of concentration of both total sugar and total amino acid of pine litter during 1977-1978 studies. The initial concentration of total sugar was almost same in the litter of both 1955 and 1970 plantations. No increase in the concentration of total sugar in decomposing litter was observed. Rather a rapid continuous fall in the concentration was measured throughout the study period (Fig.35 B).

The initial concentration of total amino acid was,

however, higher in the litter of 1955 plantation. No increase in the concentration of total amino acid was noticed at the early stage of decomposition. The concentration of total amino acid also dropped rapidly in both the case throughout the study period (Fig.36 B).

6. Types of phytotoxins released from litter during different periods of decomposition under natural condition :-

The liberation of phytotoxins from the decomposing pine needles at different time periods was studied during 1976-1977 and 1977-1978. It was observed that phytotoxins were released from the decomposing litter of all the plantations throughout the study period (Figs.40-44), but different phytotoxins were produced during different periods.of litter decomposition. 3,4 dihydroxybenzoic acid, P - coumaric acid, protocatechuic acid and Gentisik acid were produced from pine litter of 1955 plantation during 1976-1977, (Fig.40). Mostly protocatechuic acid was liberated in the early and later stages of litter decomposition but 3,4 dihydroxybenzoic acid was detected from September to December (Fig.40). Two more phytotoxins, P-coumaric acid and Gentisik acid were detected at the early stage.

3,4 dihydroxybenzoic acid, P-coumaric acid, protocatechuic acid, Gentisik acid and Caffeic acid were detected from the litter of 1965 plantation. Generally, 3,4 dihydroxybenzoic acid and protocatechuic acid were

liberated in the early and middle stage of litter decomposition. As the decomposition proceeded further caffeic acid along with protocatechuic acid were released (Fig.41).

Only three phytotoxins were detected from the decomposing pine litter of 1970 plantation, 3,4 dihydroxybenzoic acid was liberated throughout the decomposition period. P-coumaric acid was detected at the middle stage of decomposition (Fig.42).

Five phytotoxins were detected in the pine litter of 1955 plantation in the second year of investigation. P-coumaric acid and caffeic acid were frequently present during the early and middle stages of litter decomposition but as the decomposition proceeded further Protocatechuic acid and Venillic acid were liberated at the latter stages. Quinic acid was detected at the early stage (Fig.43).

Seven phytotoxins were detected from the pine litter of 1970 plantation during second year of investigation. Protocatechuic acid was detected in the early stage of decomposition. P- coumaric acid, Gentisik acid and Caffeic acid were released in the middle stage of decomposition. Quinic, P-coumaric and Venillic acids were detected at the later stage (Fig.44).

B. <u>Laboratory decomposition studies</u> :

7. <u>Morphological features of pine litter :-</u>

The physical characters, including colour and texture, of the autoclaved pine litter before inoculation

appeared to be identical with those of the original unsterilized litter.

In six months healthy mycelium of all species tested, except Sporobolomyces roseus, covered the samples of pine litter placed in glass jars on previously inoculated soil. Growth of Penicillium chrysogenum and Trichoderma viride, in particular was vigorous on litter and they spored heavily. The growth of all species tested except Sporobolomyces roseus was very prominent in soil just after one month of inoculation.

Softening and blackening of pine litter by Trichoderma viride and Penicillium chrysogenum was the most spectacular macroscopic change during the decomposition process. In six months the brown needles were changed by these fungi to white and black pulp. These changes were accompanied by some decolorization of the epidermis and removal of some dark brown tennin-like substance. Black lesions were observed on the needles after six months in all the sets inoculated with different fungi. In mixed sets the growth of both Trichoderma viride and Penicillium chrysogenum was more prominent on litter than any other fungi.

8. Dry weight loss :-

Difference in the percentage dry weight of pine leaf after various period of decomposition by the fungal species was statistically significant, (Table 76).

Changes in dry weight of pine leaf litter which occured due to the six fungal and one mixed cultures are recorded in table 81, it exhibit considerable difference

and losses ranged from less than 7 to 25% in nine months. In all cases except Absidia cylindrospora, Penicillium chrysogenum and Sporobolomyces roseus, the difference was highly significant from control.

The values for percentage dry weight of pine litter after various incubation periods with different fungal species are represented in Fig.37.

The rate of dry weight loss caused by Absidia cylindrospora and Mucor hiemalis was very rapid during first two months and it declined during the second two month periods. The relatively small change in weight caused in the last two months by these fungi can be related to their slow spread over the litter, and not more than 25% of dry weight loss was observed in nine months by these two species (Fig.37).

The rate of dry weight loss caused by other fungal species and mixed inocula was gradual during the first phase of decomposition and in no case the dry weight loss was more than 25% in nine months period (Fig.37). Less decomposition occured in litters inoculated with Penicillium chrysogenum.

Changes in chemical composition :- Changes in percentage cellulose, hemicellulose, and lignin contents of pine litter over nine months period were considered The difference in the percentage dry weight of cellulose and hemicellulose remained after various periods of

decomposition by different fungal species were statistically significant (Tables 77-78). No such significant difference was observed for lignin content of litter (Table 79).

Changes in percentage dry weight of all these three components in pine litter which occured by nine months period by different fungal species and mixed inoculum are recorded in Table 81. A 't' test was made on a comparison between the mean of the species with the percentage losses of these three components and the mean of the control. The differences in the percentage dry weight of cellulose caused by Mucor hiemalis, Trichoderma viride, Cladosporium herbarum, Sporobolomyces roseus and mixed inoculum were highly significant from control. The differences in the percentage dry weight of hemicellulose caused by Absidia cylindrospora, Trichoderma viride, Penicillium chrysogenum and mixed inoculum were also highly significant from control but no differences in the percentage dry weight of lignin content by different inoculum was observed from control (Table 81).

The loss of cellulose was least in the control sets than the inoculated sets (Fig.38). The loss of cellulose caused by different species was very rapid during the first three months of decomposition. Subsequently, decrease cellulose content was gradual. The highest loss in cellulose content was observed in the litter inoculated with Mucor hiemalis and was followed by mixed culture (Fig.38).

The loss of lignin in nine months period was

least in control than inoculated sets (Fig.38). A gradual loss in lignin was observed in all the sets and the highest loss was observed in the litter inoculated with Trichoderma viride (Fig.38). Sporobolomyces roseus and mixed inoculum followed next.

A gradual loss in hemicellulose was observed during the first three months before declining rapidly in later stages. The loss in hemicellulose due to different fungal species was appreciably high and after nine months the loss was 11%. This loss was relatively less in litter inoculated with Trichoderma viride (Fig.38). Significant differences were observed in the loss of hemicellulose in the litter inoculated with Absidia, Trichoderma, Penicillium and mixed inoculum than the control (Fig.81).

However, loss of all these three constituents from pine litter followed total weight loss very closely.

9. Hydrogen ion concentration :- The pine leaf litter was less acidic during first six months of decomposition but the pH value decreased further below 4.0 after nine months in all irrespective of inoculated and noninoculated sets.

10. CO_2 evolution from decomposing litter :- Co_2 evolution from pine litter inoculated with different fungal species and during different period of decomposition was recorded.

The differences in the CO_2 evolution from decomposing pine litter inoculated with different fungal species were statistically significant, the variation in

Co_2 evolution during different periods of decomposition was also statistically significant (Table 80).

Changes in Co_2 evolution from decomposing litter which occured in relation to different fungal cultures and in mixed inoculum are recorded in table 81. Considerable difference was observed in the Co_2 evolution from decomposing litter inoculated with different fungal species. A 't' test was made on a comparison between the mean of Co_2 evolved from inoculated sets with those of controls. In all cases except *Absidia cylindrospora* and *Mucor hiemalis* the difference was highly significant from control (Table 81 and Fig.39). No significant correlation was observed in the Co_2 evolution and the soil temperature as well as Co_2 evolution and dry weight loss of pine litter.

11. Phytotoxins released during decomposition of pine litter by different fungal species :-

From the laboratory decomposition studies of pine litter it has been found that during first three months of litter decomposition different types of phytotoxins were released from litter inoculated with different fungi (Figs. 45-47). No phytotoxins were detected from the litter leachates after 90 days of decomposition. Phytotoxins were, however, detected from the control sets also.

3,4 dihydroxybenzoic acid and caffeic acid were detected from the uninoculated control sets during the first month of decomposition. Protocatechuic, caffeic and quinic acids were detected from the leachates of pine

litter inoculated with Mucor hiemalis. 3,4 dihydroxybenzoic acid, caffeic and gentisik acid were detected from the leachates of pine litter inoculated with Absidia cylindrospora and Trichoderma viride. 3,4 dihydroxybenzoic acid, Protocatechuic, P-coumaric and quinic acid were detected from leachates of pine litter inoculated with Cladosporium herbarum. Litter inoculated with Penicillium chrysogenum produced only caffeic acid in the first month of decomposition. Protocatechuic acid was detected from leachates of pine litter inoculated with Sporobolomyces roseus and mixed inoculum.

During the second month of litter decomposition protocatechuic acid was detected only from control sets. 3,4 dihydroxybenzoic acid and gentisik acid was detected from Mucor inoculated set. No toxins could be detected in the leachates of Absidia inoculated litter in second month. 3,4 dihydroxybenzoic acid, venillic and quinic acids were released in the litter inoculated with Penicillium chrysogenum. Protocatechuic, ferulic, gentisik, quinic acids were detected in the pine litter inoculated with Cladosporium herbarum. 3,4 dihydroxybensoic, ferulic, protocatechuic, gentisik and caffeic acids were detected from the litter inoculated with Trichoderma viride. Sporobolomyces roseus and mixed inoculum respectively. During the first two months of decomposition highest number of phytotoxins were detected from the litter inoculated with Cladosporium herbarum, also maximum number of toxins were produced during these periods (Figs.45-47).

During the third month of litter decomposition very less number of toxins were produced. Only four phytotoxins were produced during this period from the different sets. 3,4 dihydroxybenzoic acid was very common and was detected from almost all leachates (Fig.47).

Figure - 21 : Seasonal variation and correlation in fungal population and moisture content of decomposing pine leaf litter from litter bags kept on the forest floor of three different plantations during 1976-1977.

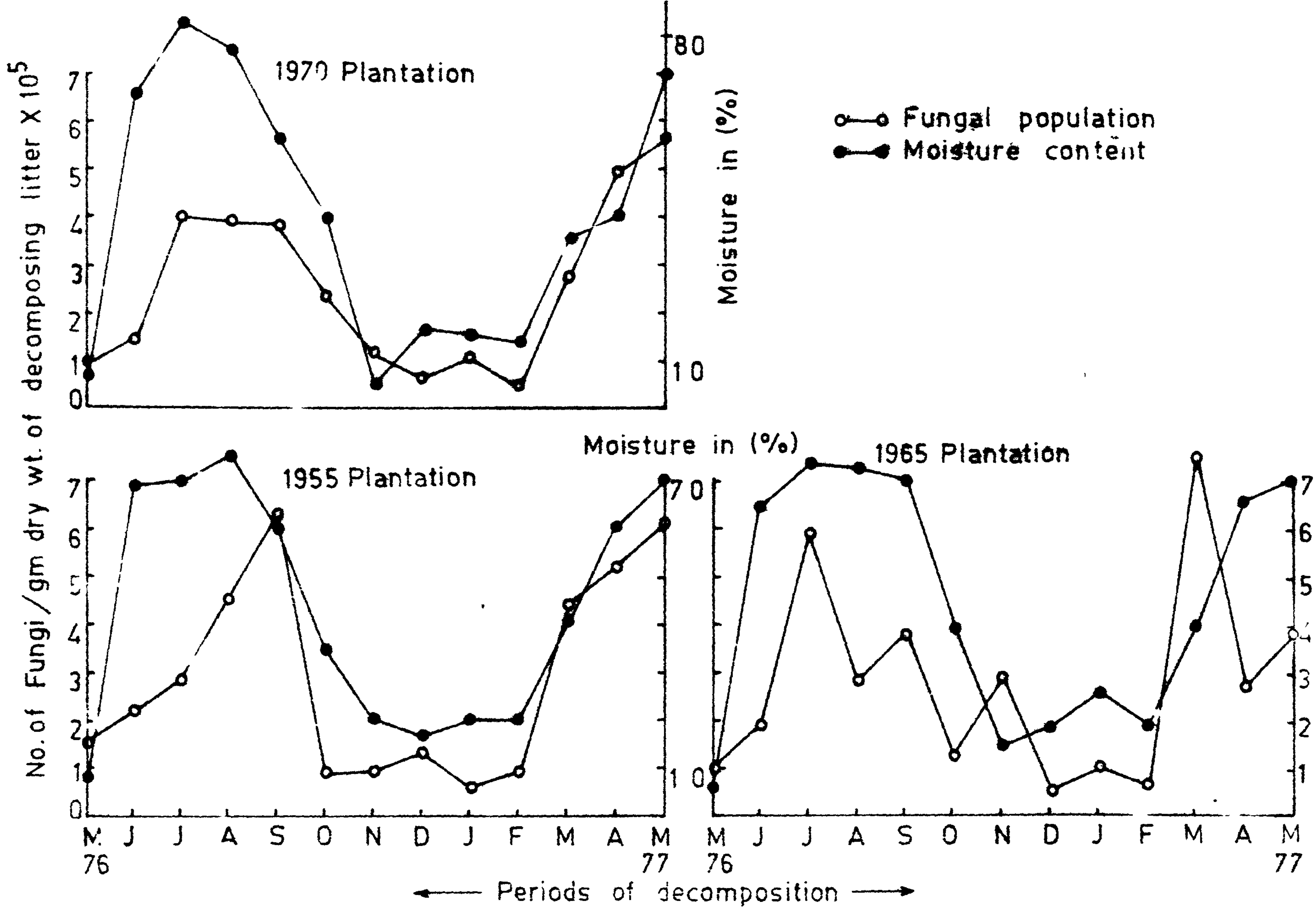


Figure 21

Figure - 22 : Seasonal variation and correlation in bacterial population and moisture content of decomposing pine leaf litter from litter bags kept on the forest floor of three different plantations during 1976-1977.

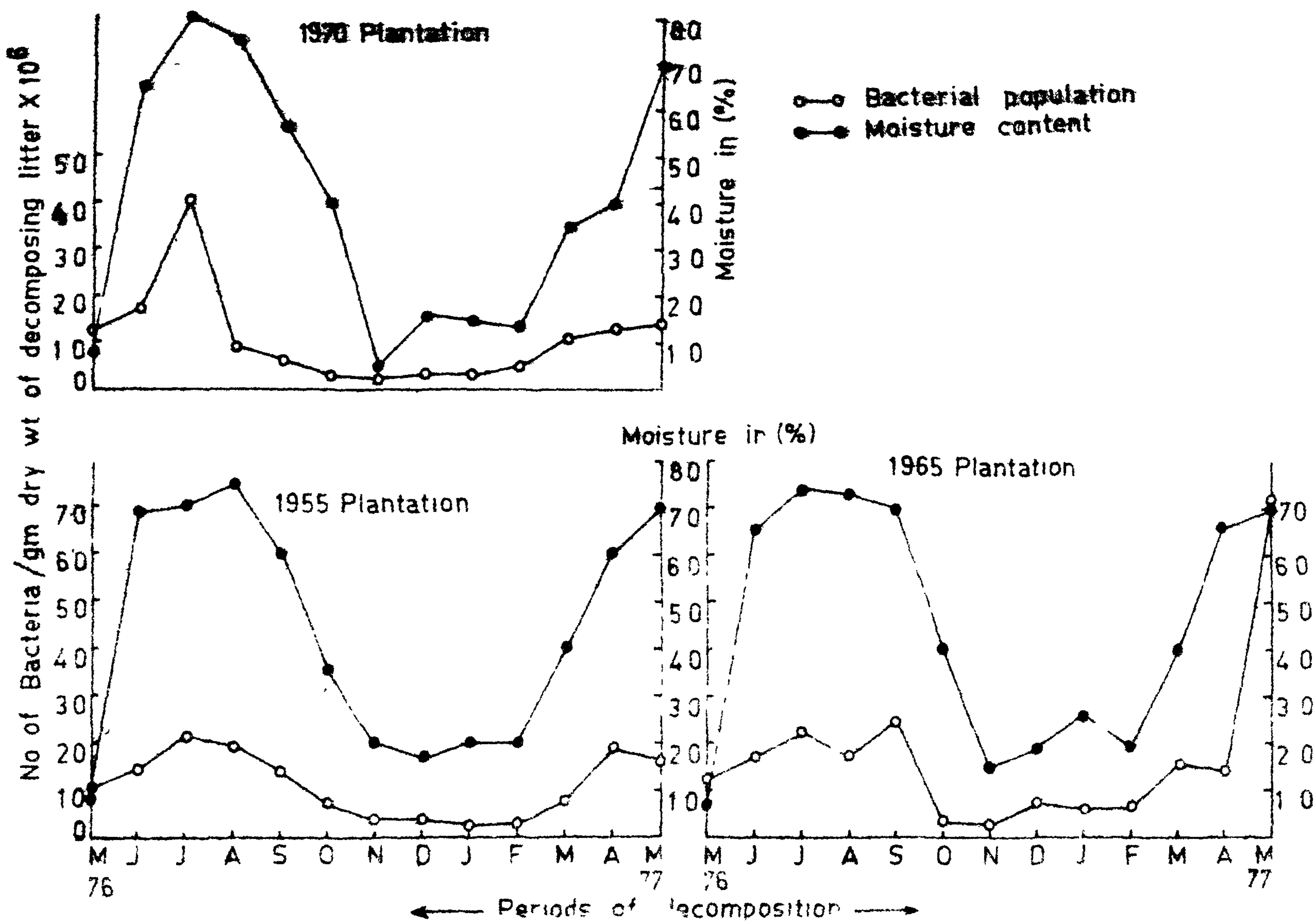


Figure 22

Figure - 23 : Seasonal variation and correlation in actinomycetes population and moisture content of decomposing pine leaf litter from litter bags kept on the forest floor of three different plantations during 1976-1977.

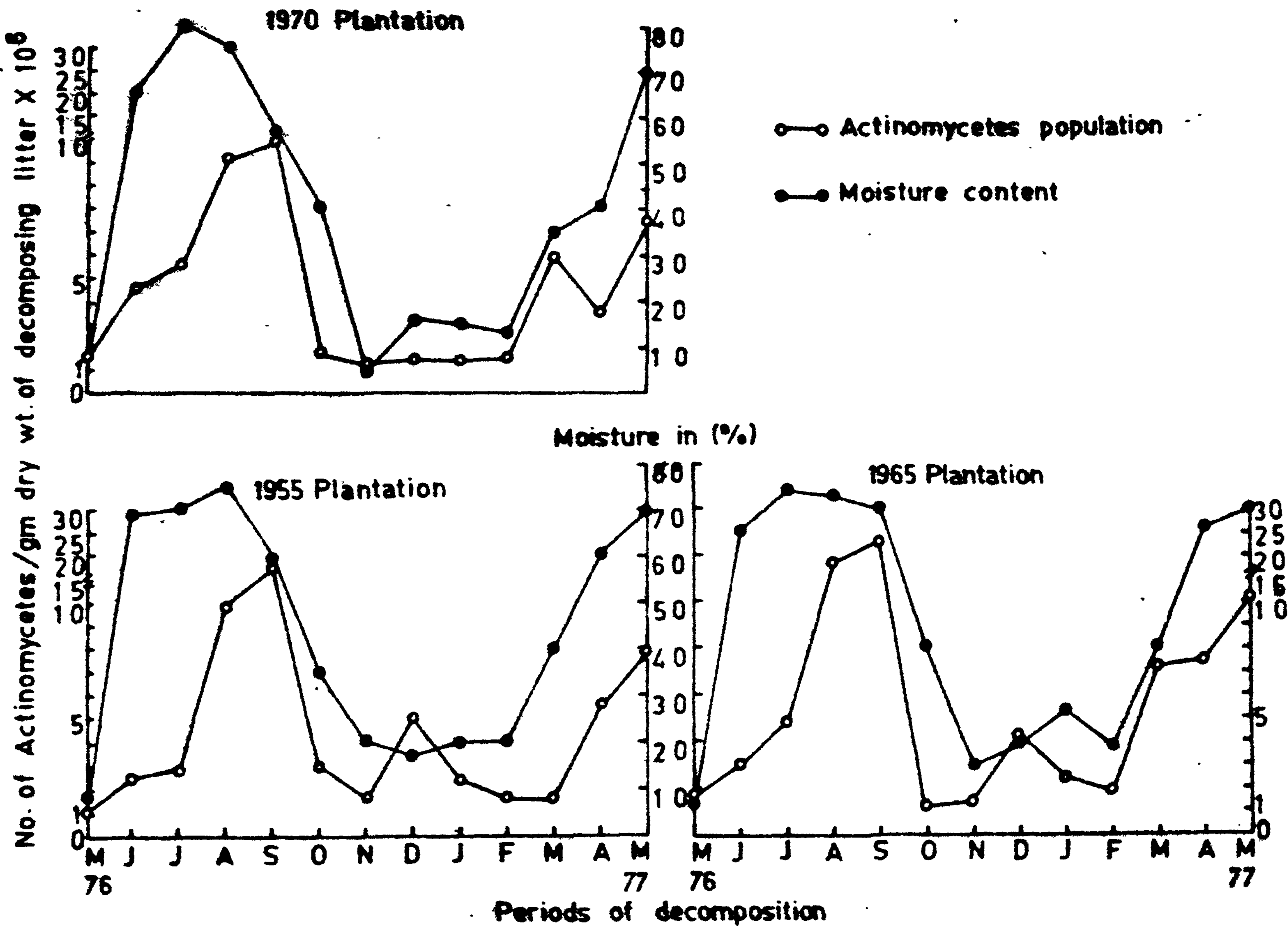


Figure 23

Figure - 24 : Seasonal variation and correlation in fungal, bacterial, actinomycetes population and moisture content of decomposing pine leaf litter from litter bags kept on the forest floor of 1970 plantation during 1977-1978.

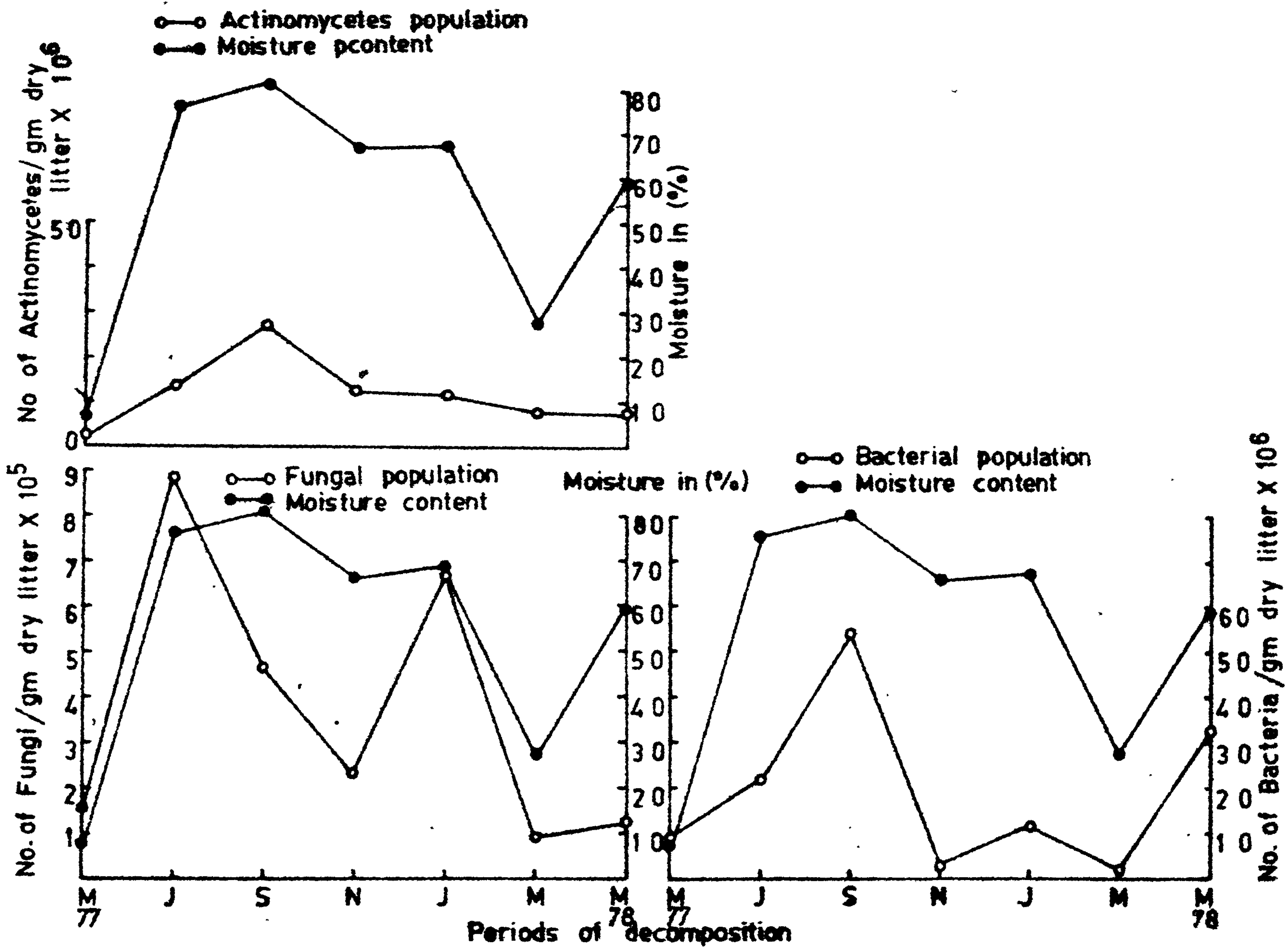


Figure 24

Figure - 25 : Histograms showing the percentage relative abundance of various fungal species occured on pine leaf litter of 1955 plantation at various periods of decomposition during 1976-1977.
1 = *Mucor hiemalis*, 2 = *Pythium* sp., 3 = *Absidia cylindrospora*, 4 = *Circinella* sp., 5 = *Aspergillus* sp., 6 = *Penicillium chrysogenum*, 7 = *Aureobasidium pullulans*, 8 = Unidentified ascomycetes, 9 = *Cladosporium herbarum*, 10 = *Trichoderma viride*, 11 = *Verticillium* sp., 12 = Sterile white mycelia.

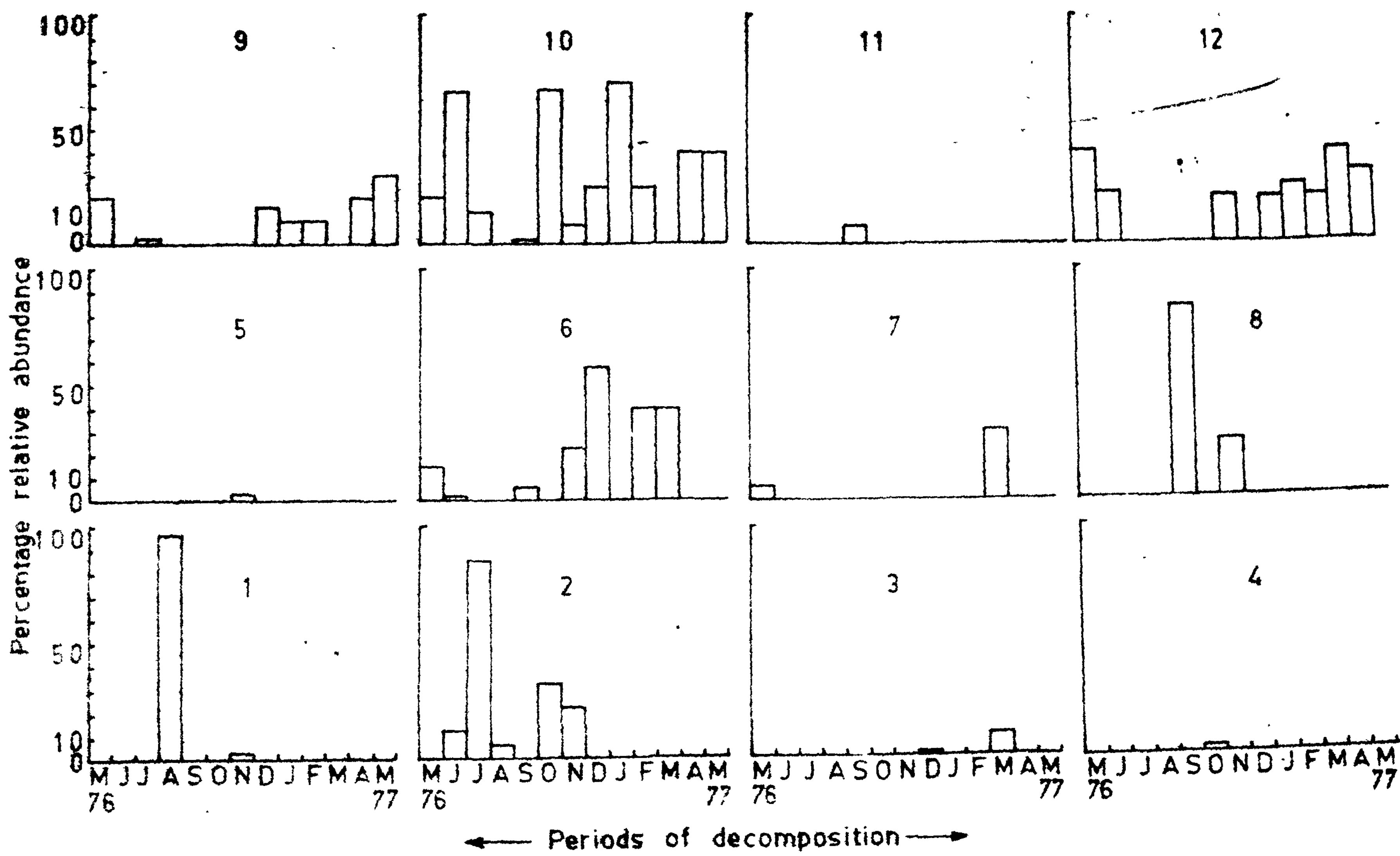


Figure 25

Figure - 26 : Histograms showing the percentage relative abundance of various fungal species occured on pine leaf litter of 1965 plantation at various periods of decomposition during 1976-1977.

1 = *Mucor hiemalis*, 2 = *Pythium* sp., 3 = *Absidia cylindrospora*, 4 = *Penicillium chrysogenum*, 5 = *Aureobasidium pullulans*, 6 = *Geotrichum* sp., 7 = *Sporobolomyces roseus*, 8 = *Trichoderma viride*, 9 = *Cladosporium herbarum*, 10 = *Verticillium* sp, 11 = *Fusarium sporotrichoides*, 12 = Sterile white mycelia.

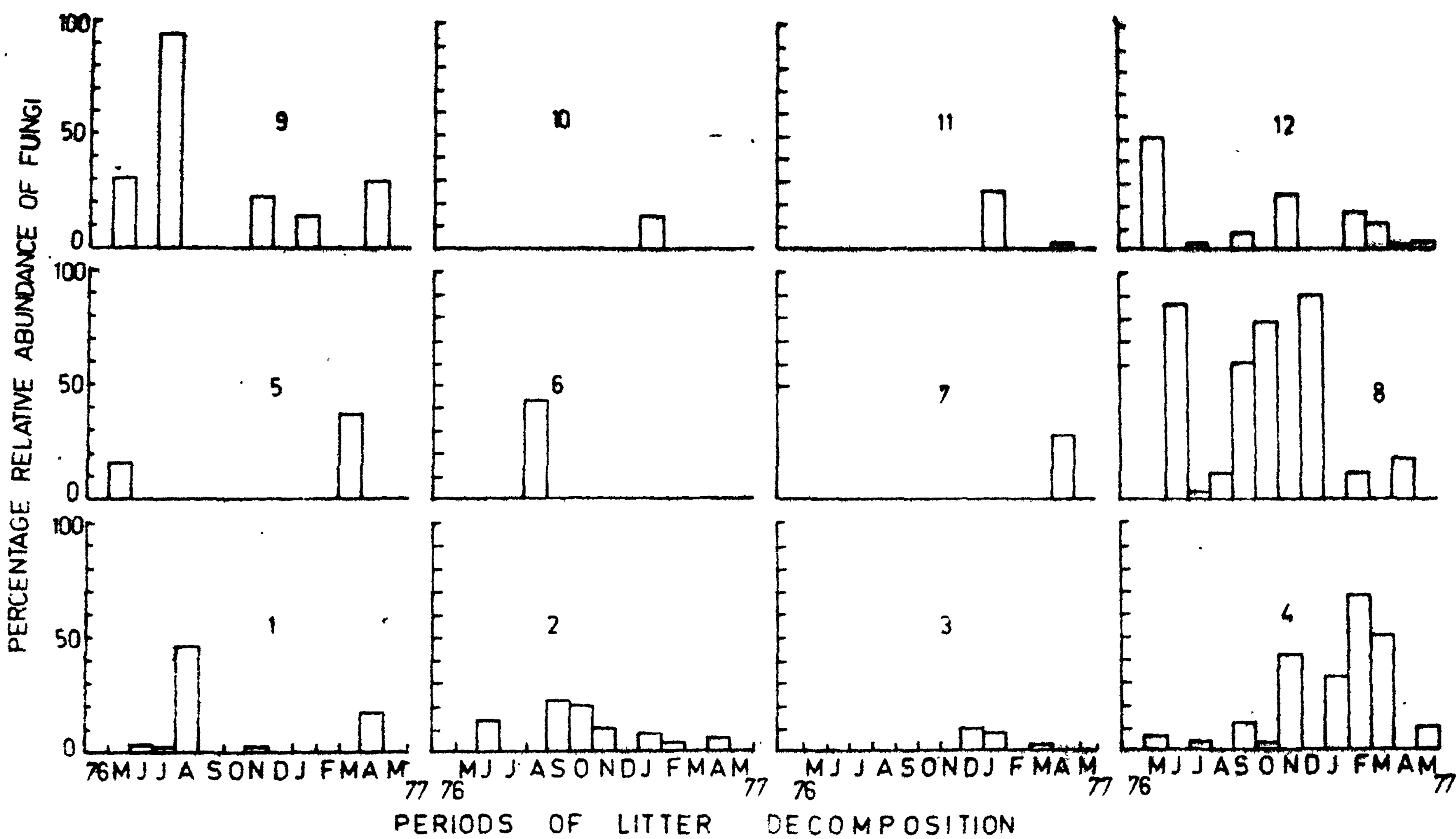


Figure 26

Figure - 27 : Histograms showing the percentage relative abundance of various fungal species occured on pine leaf litter of 1970 plantation at various periods of decomposition during 1976-1977.

1 = *Mucor hiemalis*, 2 = *Pythium* sp., 3 = *Absidia cylindrospora*, 4 = *Cladosporium herbarum*, 5 = *Penicillium chrysogenum*, 6 = *Aureobasidium pullulans*, 7 = *Trichoderma viride*, 8 = *Verticillium* sp., 9 = *Fusarium sporotrichoides*, 10 = *Stemphylium* sp., 11 = Sterile white mycelia, 12 = *Curvularia* sp.

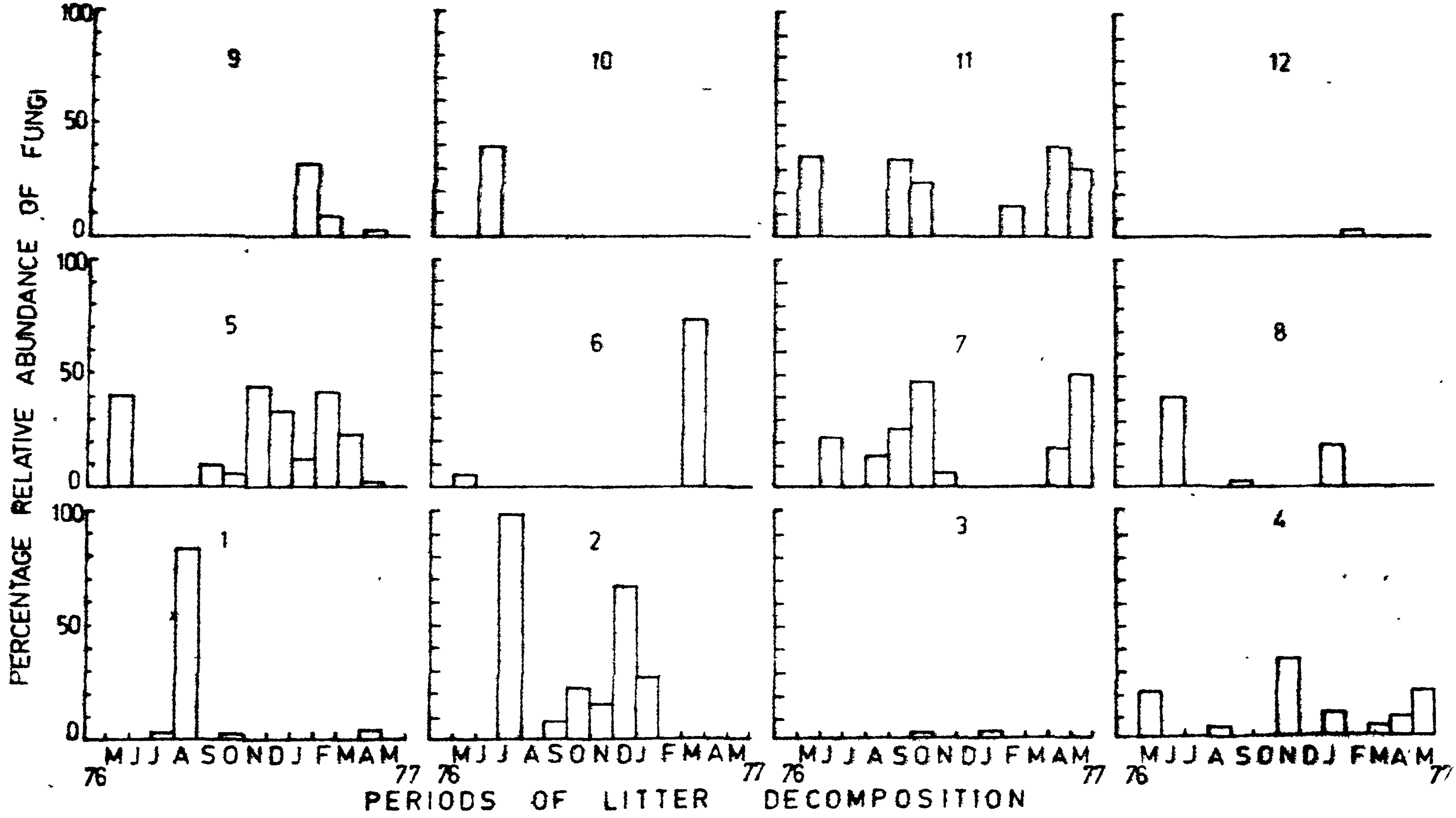


Figure 27

Figure - 28 : Histograms showing the percentage relative abundance of various fungal species occured on pine leaf litter of 1970 plantation at various periods of decomposition during 1977-1978.

1 = *Mucor hiemalis*, 2 = *Pythium* sp.,
3 = *Cladosporium herbarum*,
4 = *Sporobolomyces roseus*,
5 = *Cladosporium herbarum*,
6 = *Trichoderma viride*,
7 = *Fusarium sporotrichoides*,
8 = *Phoma humicola*, 9 = Sterile white mycelia.

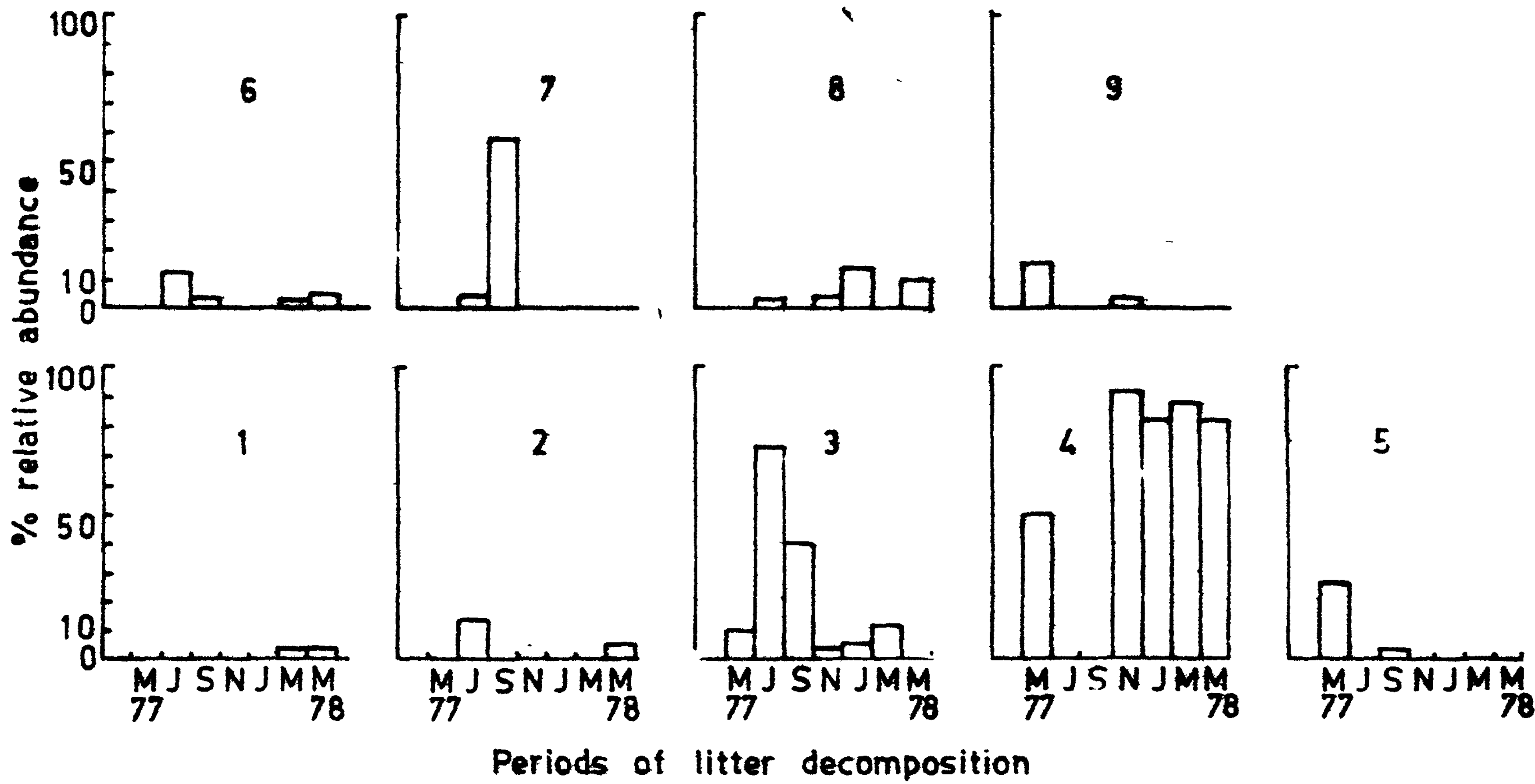


Figure 28

Figure - 29 : Percentage of the original dry weight of pine leaf litter remaining after various periods of decomposition on the forest floor of three different plantations during 1976-1977 (Nylon bag technique).

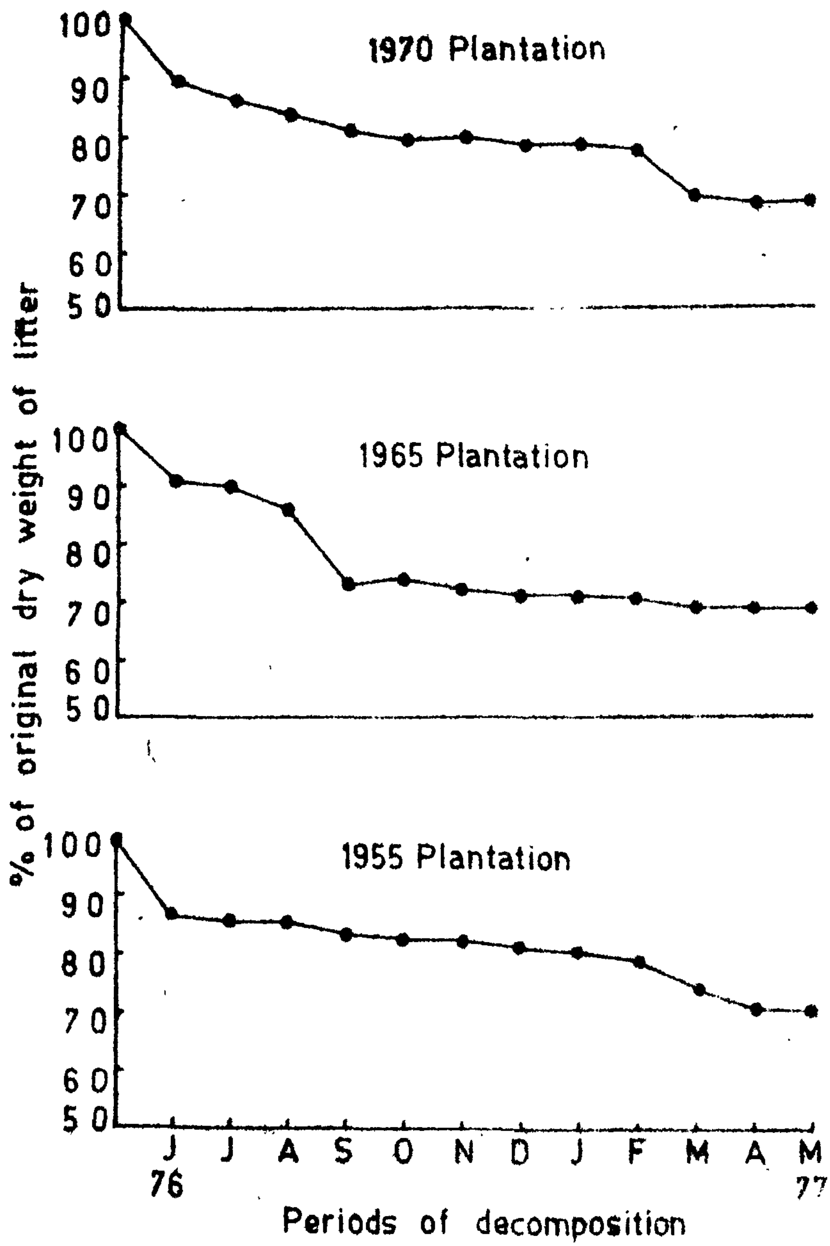


Figure 29

Figure - 30 : Percentage of the original dry weight of pine leaf litter remaining after various periods of decomposition on the forest floor of two different plantations during 1977-1978 (nylon bag technique).

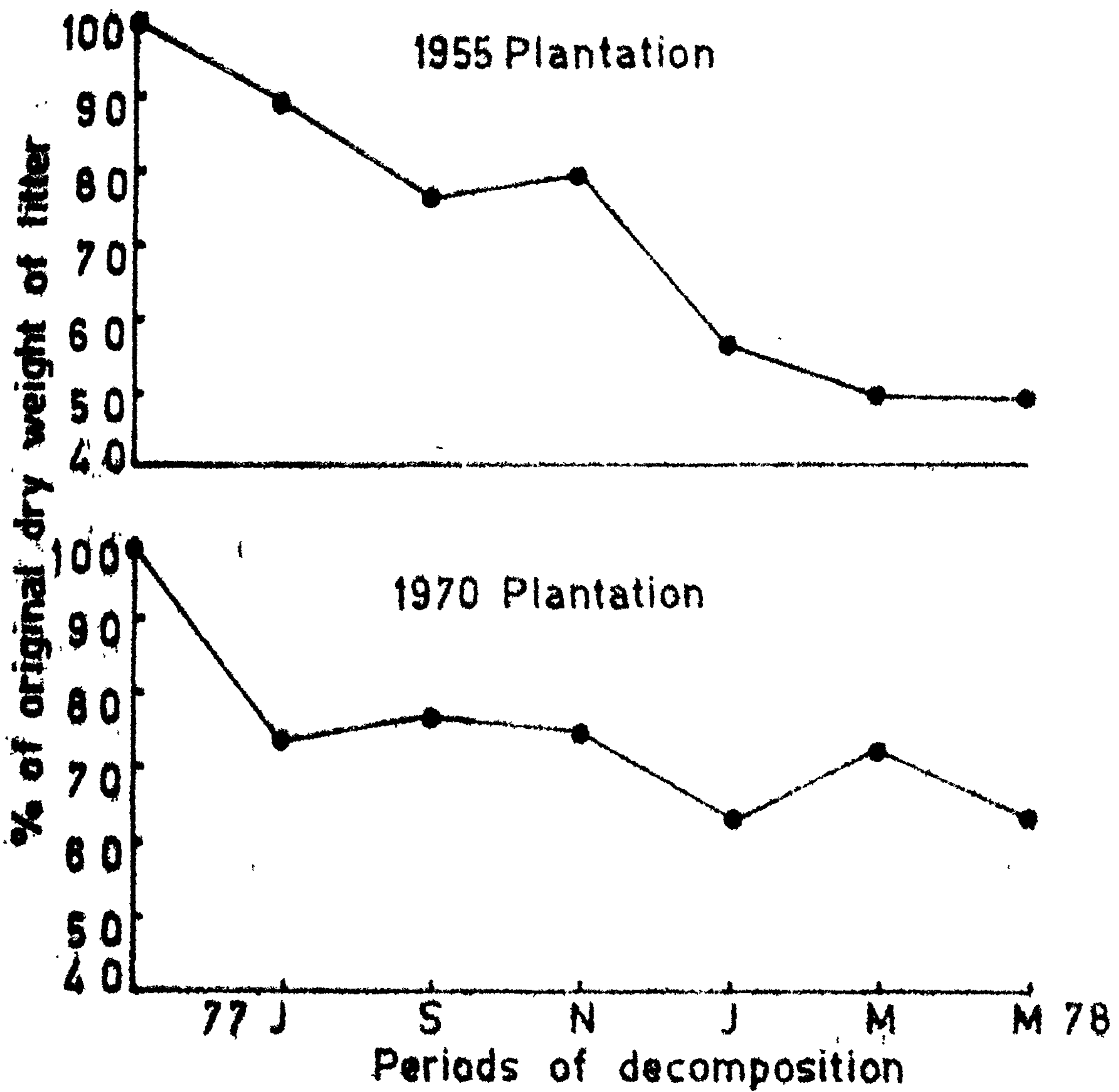


Figure 30

Figure - 31 : Showing course of change of pH and moisture content of pine leaf litter kept on the forest floor of three different plantations, at various periods of decomposition.

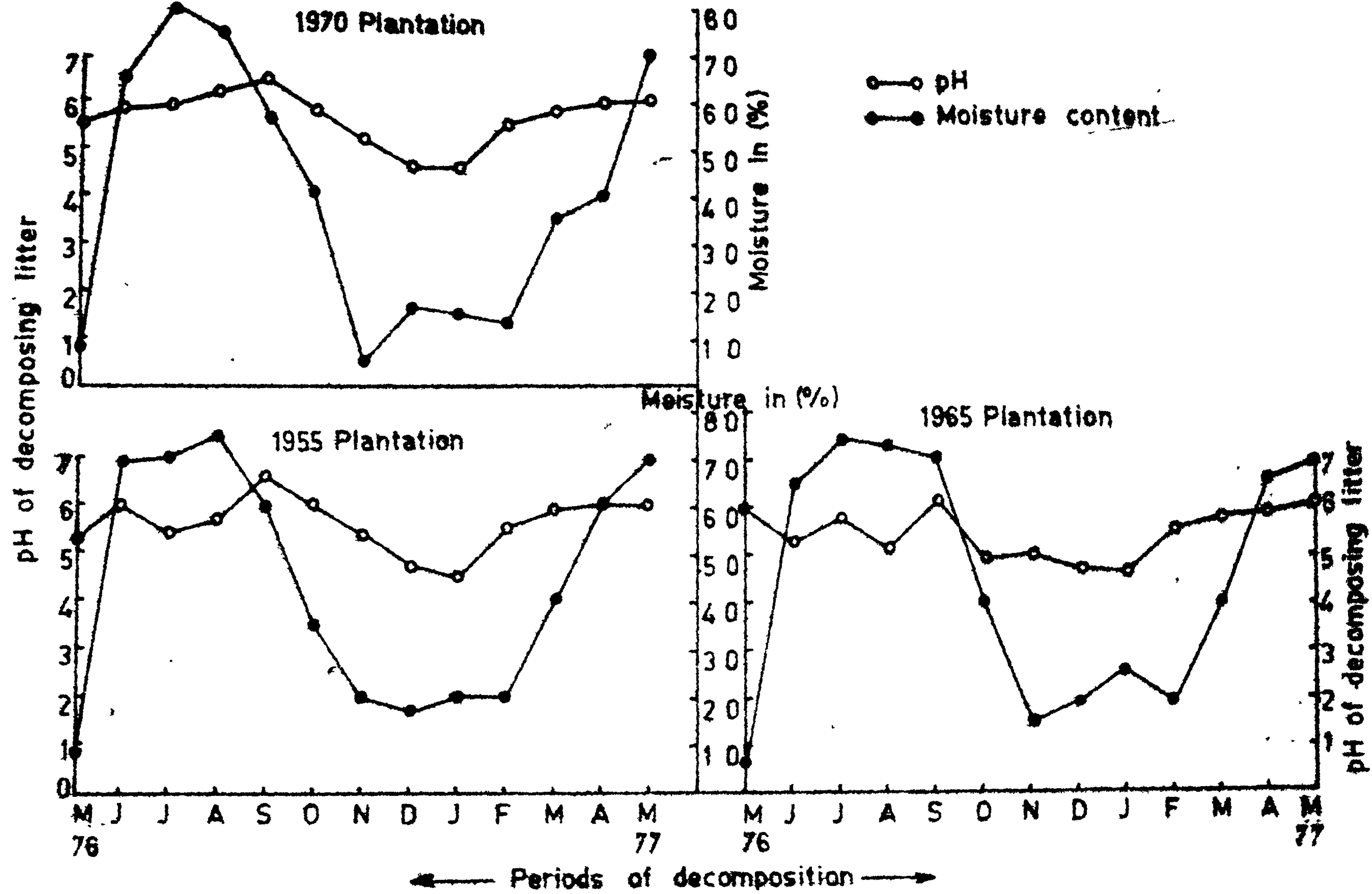


Figure 31

Figure - 32 : Relationship between total number of microbes, carbondioxide, evolution and moisture content of pine litter of 1970 plantation at various periods of decomposition during 1977-1978.

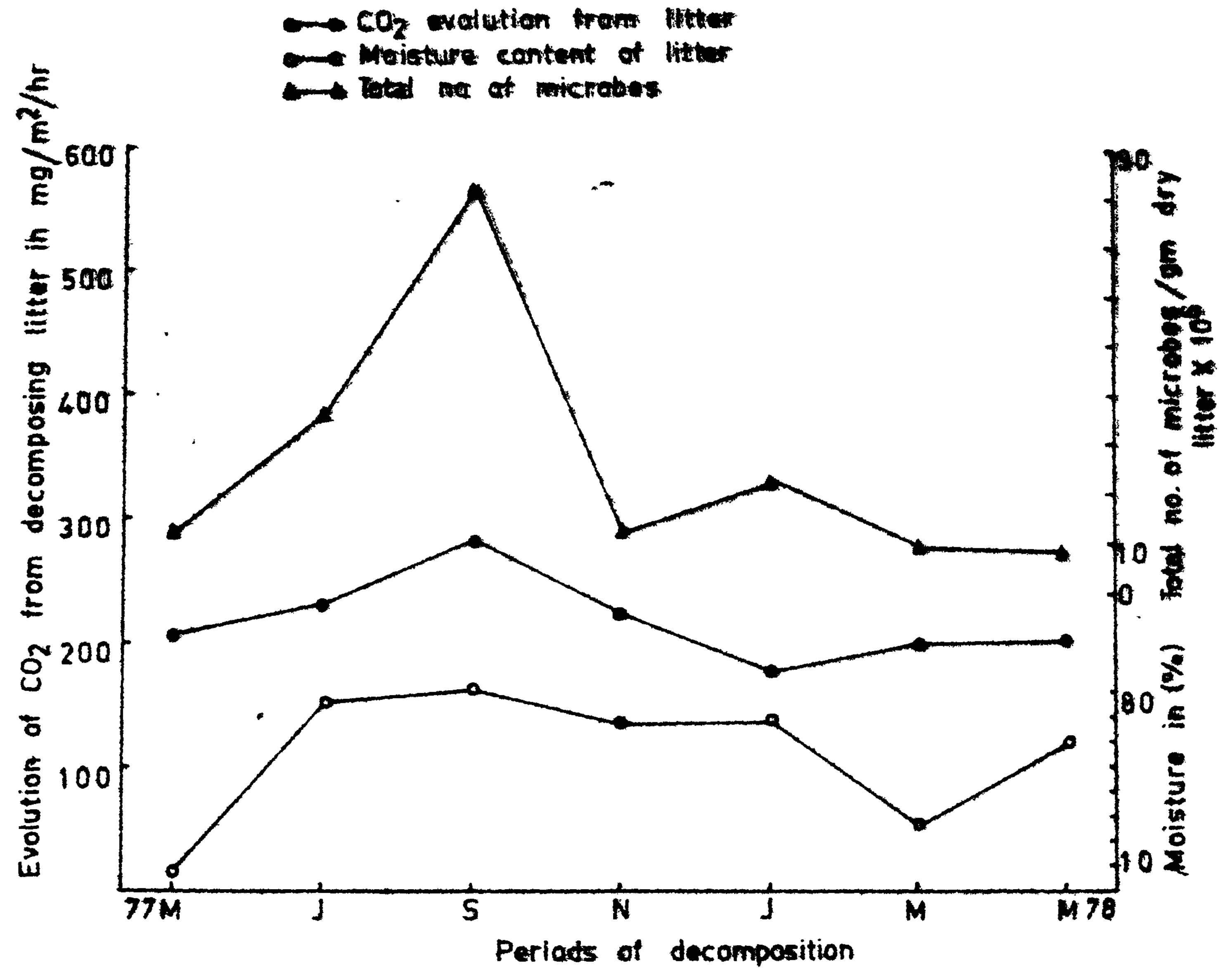


Figure 32

Figure - 33 : Histograms showing percentage of cellulose, lignin and hemicellulose of pine leaf litter remaining after various periods of decomposition on the forest floor of three different plantations during 1976-1977.

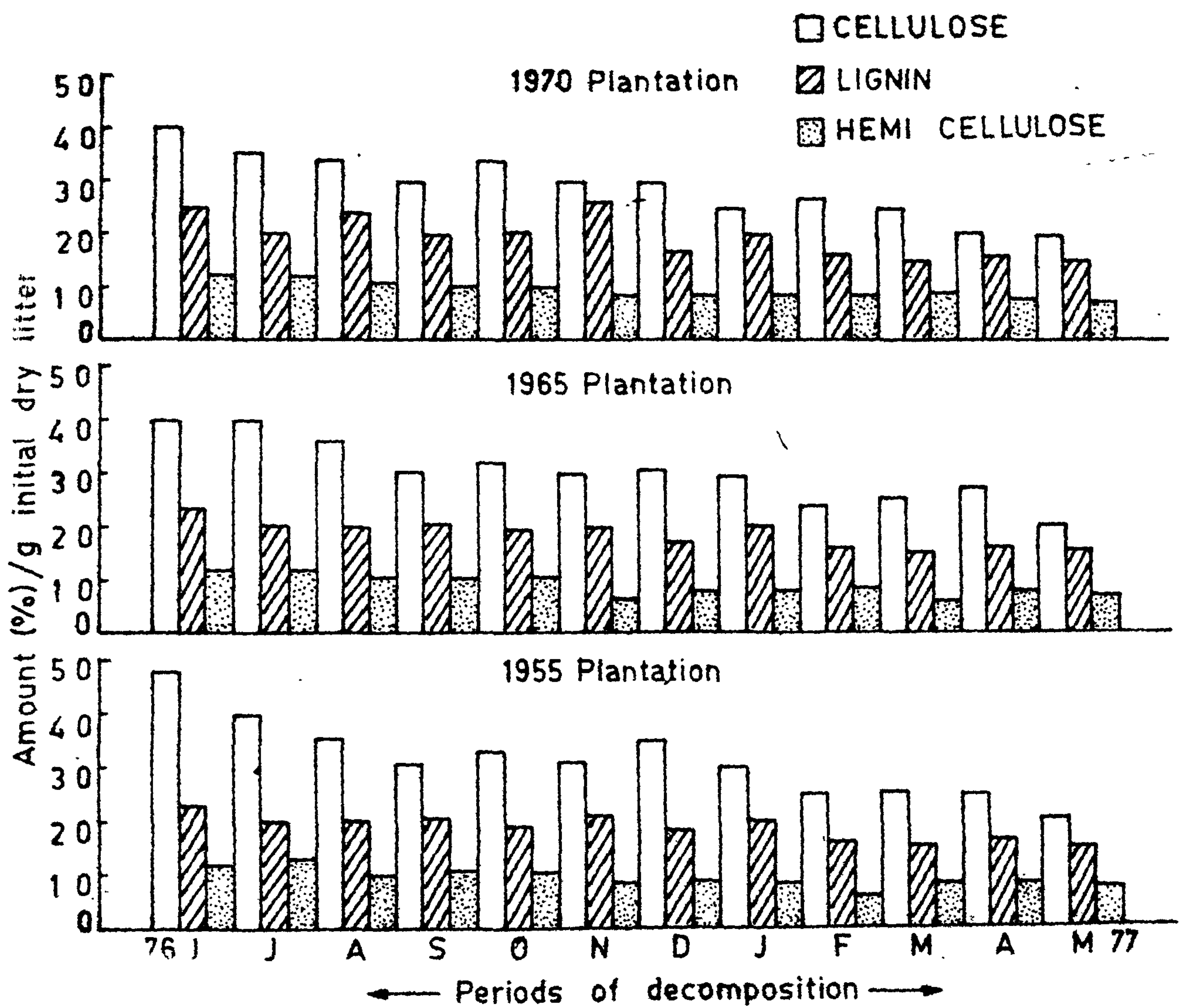


Figure 33

Figure - 34 : Histograms showing percentage cellulose, lignin and hemicellulose of pine leaf litter remaining after various periods of decomposition on the forest floor of two different plantations during 1977-1978.

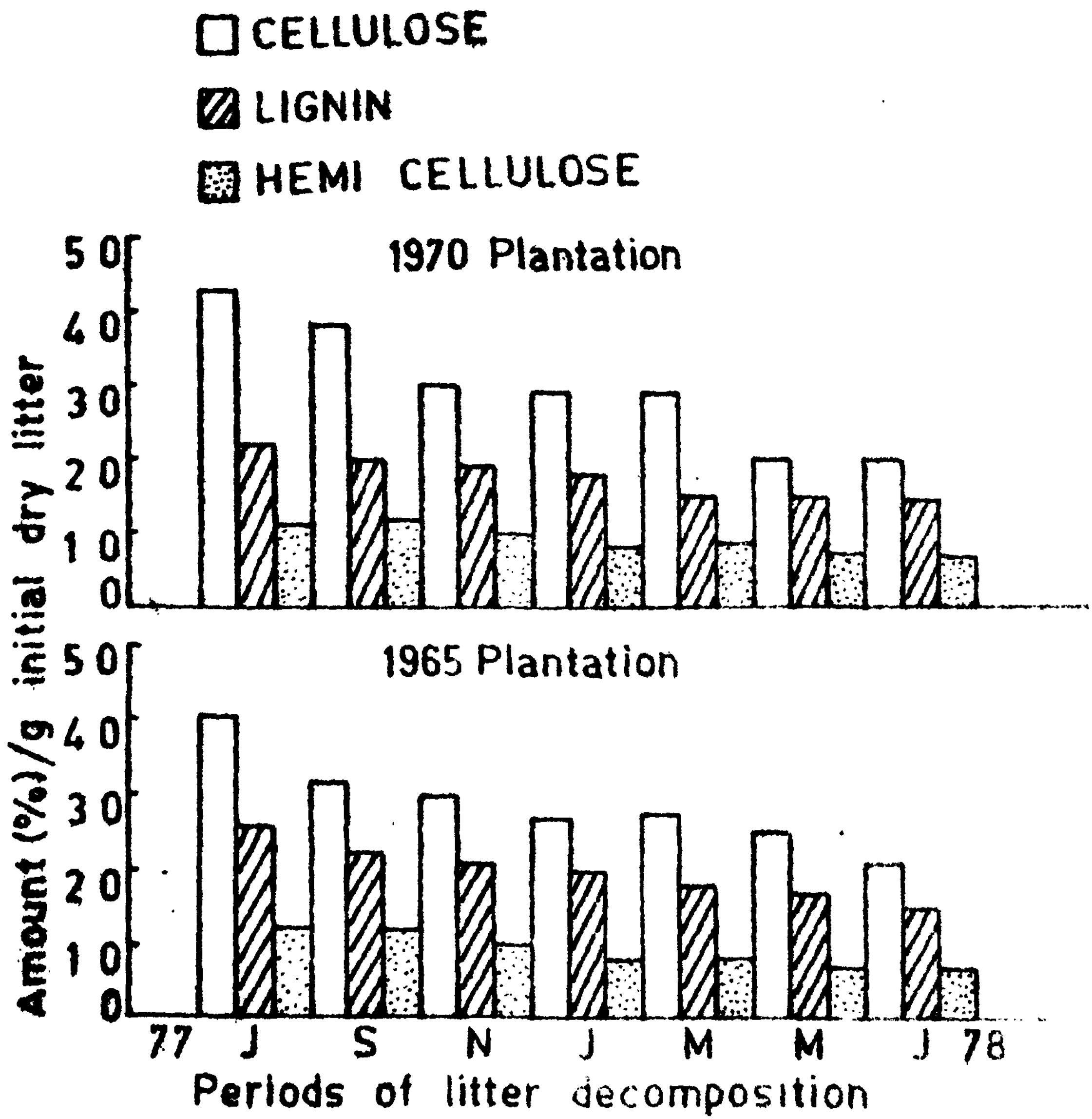


Figure 34

Figure - 35 : (A) Showing concentration of total sugar of pine leaf litter of three different plantation remaining after various period of decomposition during 1976-1977.

(B) Showing concentration of total sugar of pine leaf litter of only two plantations remaining after various period of decomposition during 1977-1978.

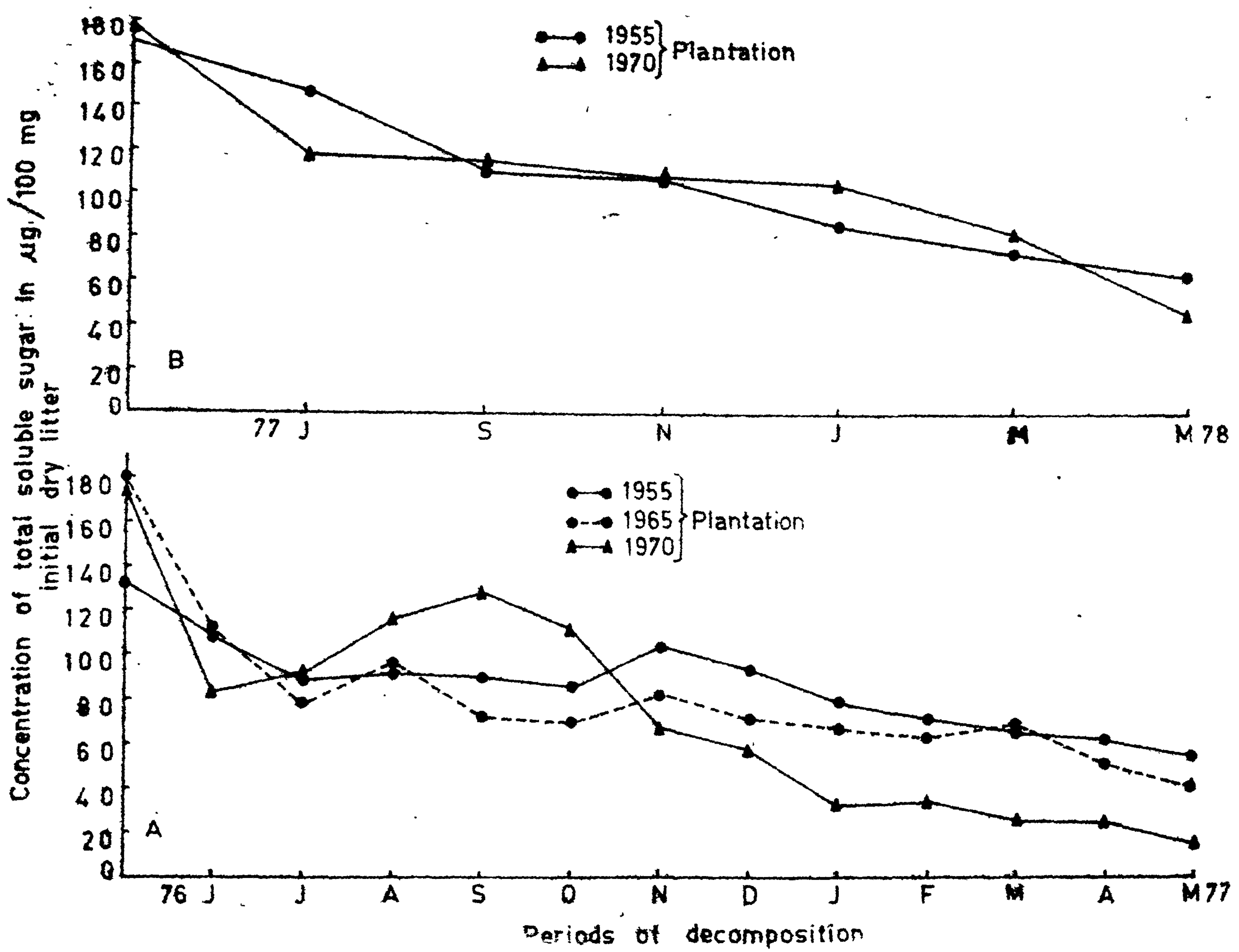


Figure 35

Figure - 36 : (A) Showing concentration of total amino acid of pine leaf litter of three different plantations remaining after various periods of decomposition during 1976-1977.

(B) Showing concentration of total amino acid of pine leaf litter of only two plantations remaining after various periods of decomposition during 1977-78.

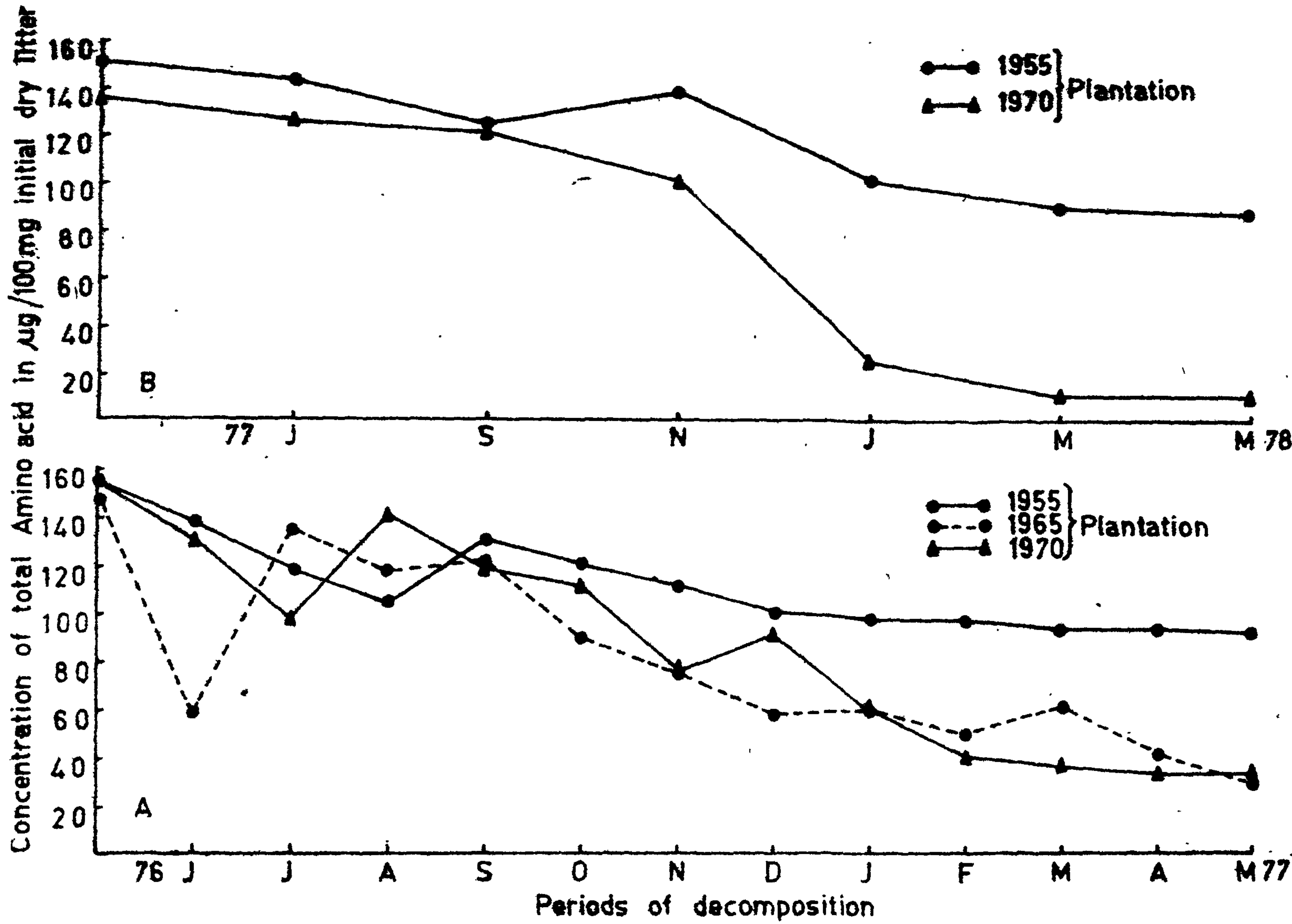


Figure 36

Figure - 37 : Showing percentage of original dry weight of pine leaf litter remaining after various period of decomposition by certain selected fungal species under laboratory condition.

Selected fungal species : 1=Control, 2 = *Absidia cylindrospora*, 3 = *Mucor hiemalis*, 4 = *Trichoderma viride*, 5 = *Penicillium chrysogenum*, 6 = *Cladosporium herbarum*, 7 = *Sporobolomyces roseus*, 8 = Mixed inoculum.

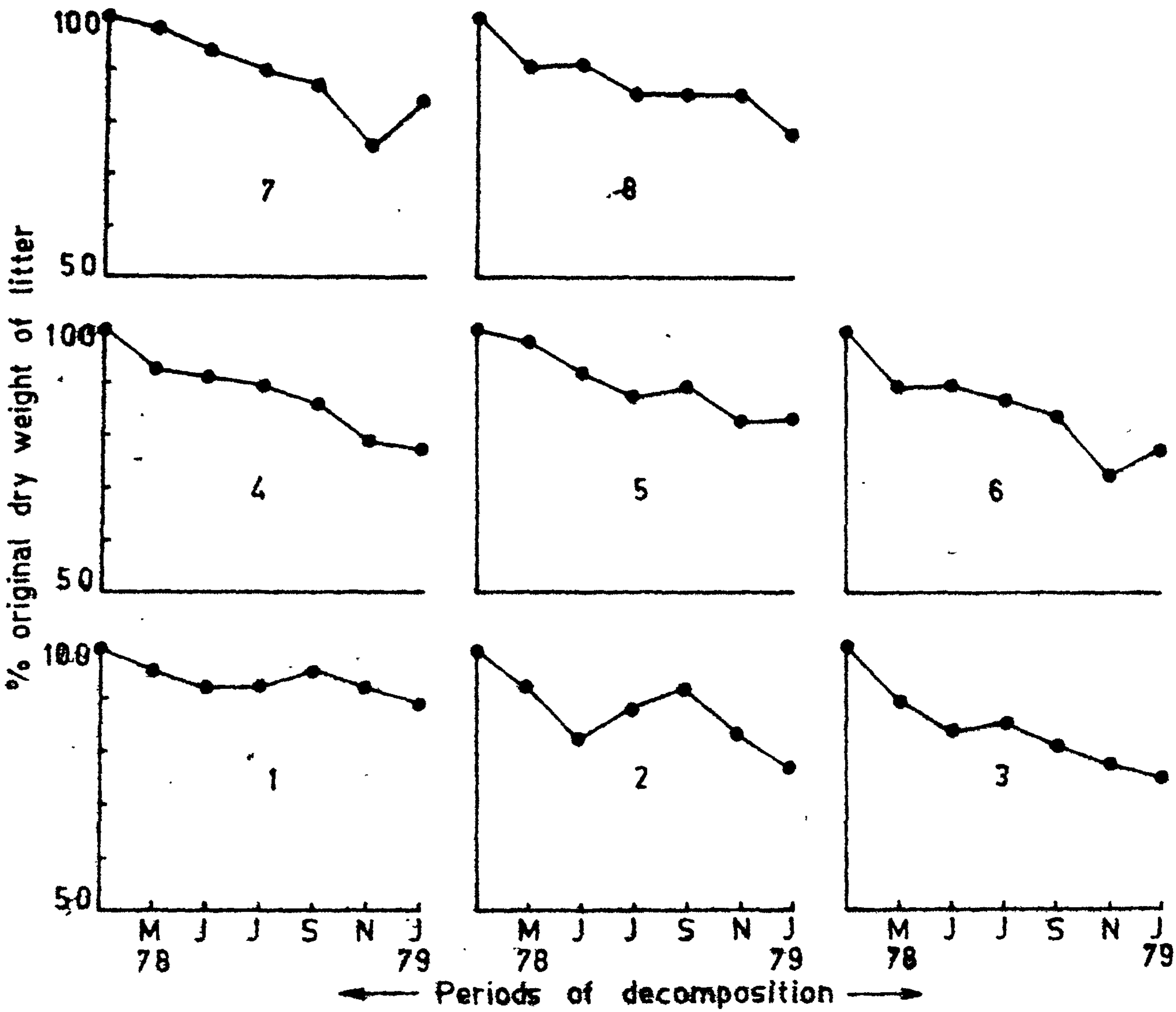


Figure 37

Figure - 38 : Showing percentage of cellulose, hemicellulose and lignin remaining in pine litter after various periods of decomposition by certain selected fungal species under laboratory condition.

Selected fungal species : 1=Control
2 = *Absidia cylindrospora*, 3=*Mucor hiemalis*, 4 = *Trichoderma viride*,
5 = *Penicillium chrysogenum*,
6 = *Cladosporium herbarum*,
7 = *Sporobolomyces roseus*, 8
8 = Mixed inoculum.

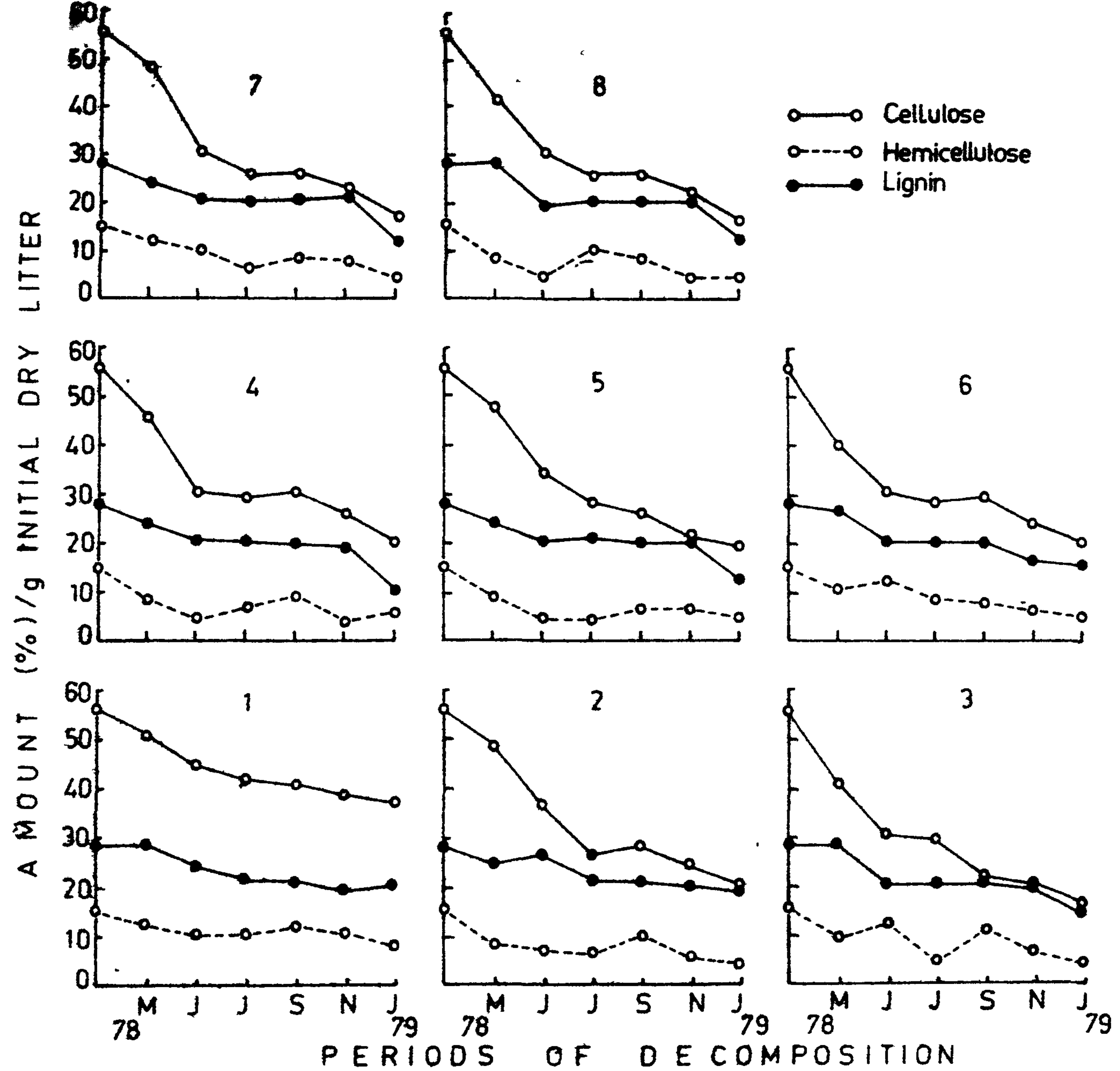


Figure 38

Figure - 39 : Showing evolution of CO_2 from pine litter at various periods of decomposition by certain selected fungal species under laboratory condition.

Selected fungal species : 1=Control, 2 = *Absidia cylindrospora*, 3=*Mucor hiemalis*, 4 = *Trichoderma viride*, 5 = *Penicillium chrysogenum*, 6 = *Cladosporium herbarum*, 7 = *Sporobolomyces roseus* 8 = Mixed inoculum.

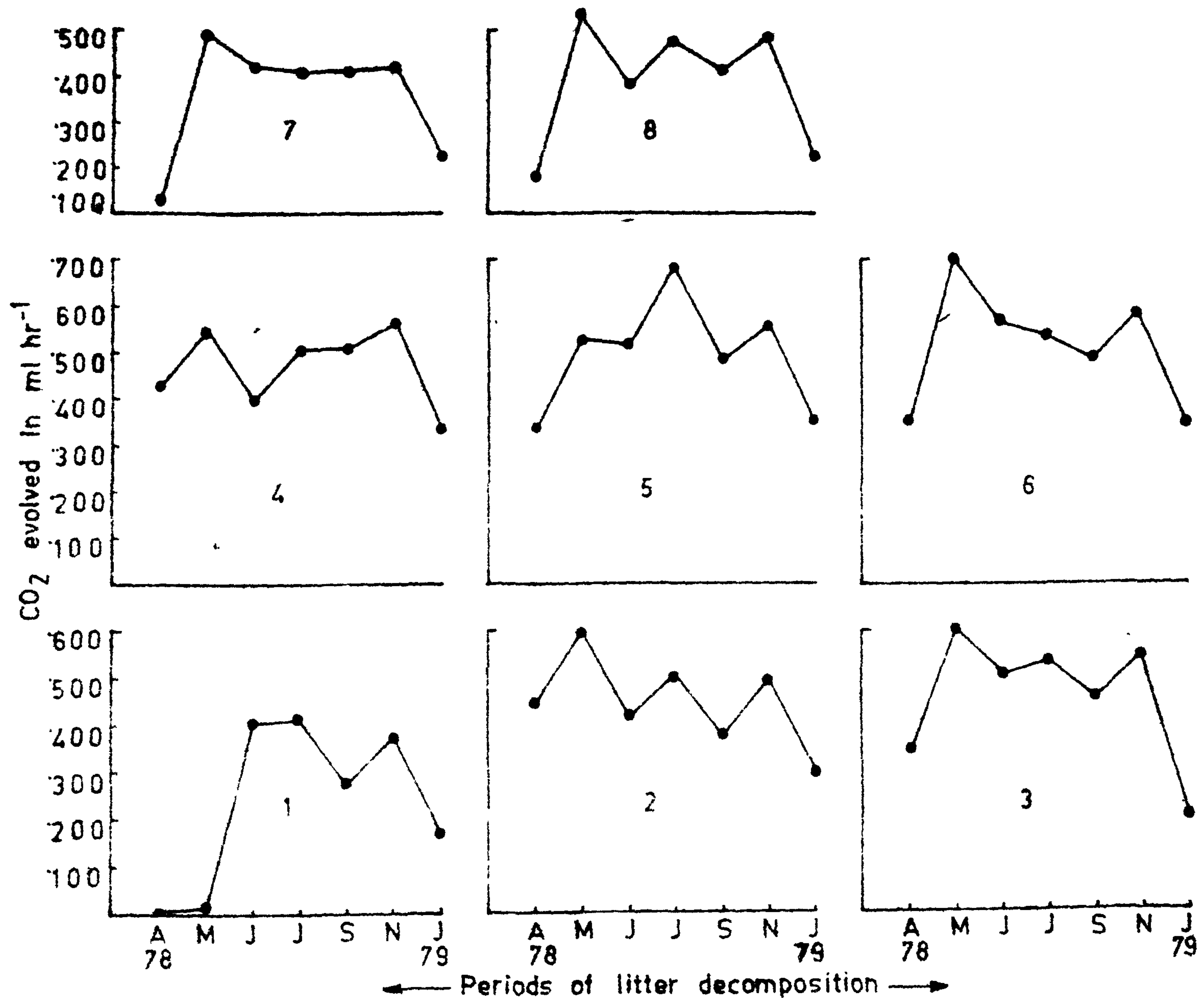


Figure 39

Figure - 40 : Chromatogram showing the presence of various phytotoxins in leachates of decomposing pine litter.

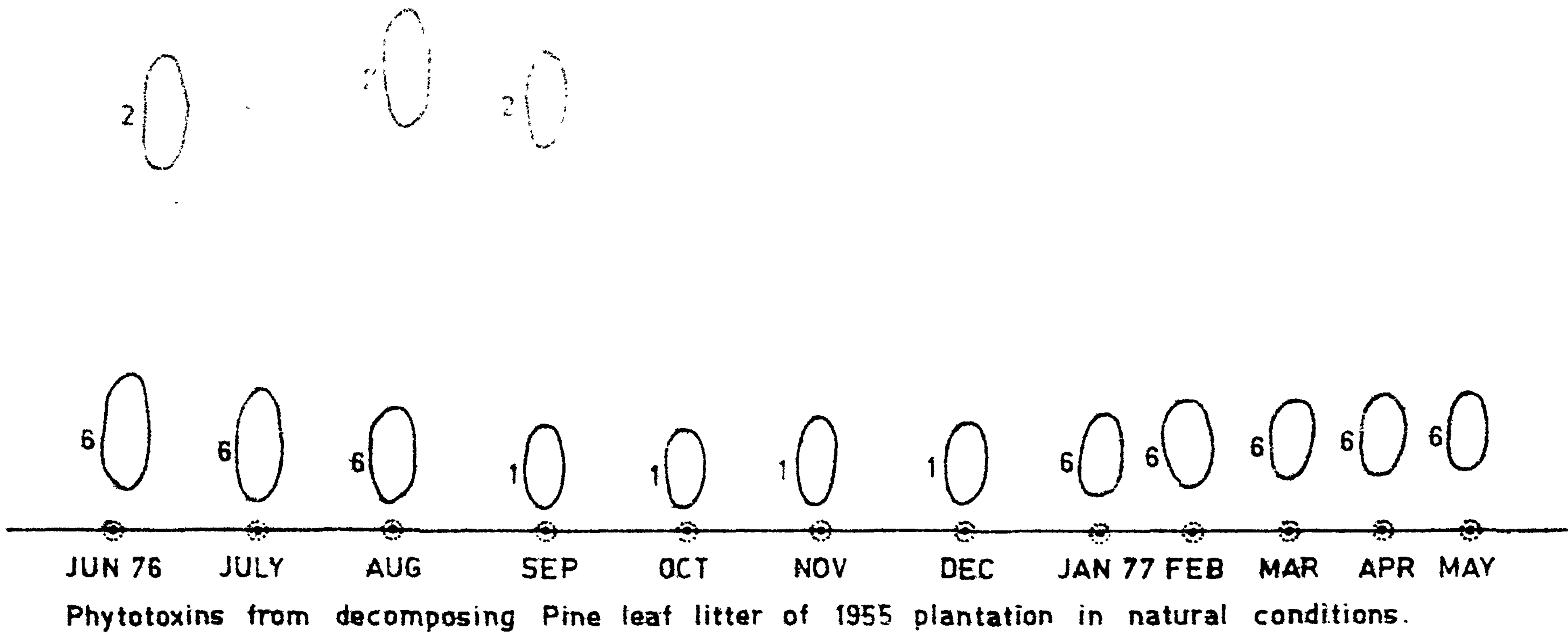
<u>Phytotoxins</u> :

1 = 3,4 dihydroxybenzoic acid.

2 = p, coumaric acid

5 = protocatechuic acid

7 = Gentisic acid.



Phytotoxins from decomposing Pine leaf litter of 1955 plantation in natural conditions.

Figure 40

Figure - 41 : Chromatogram showing the presence of various phytotoxins in leachates of decomposing pine litter.

<u>Phytotoxins</u> :

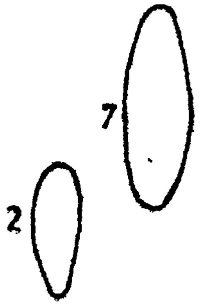
1 = 3,4 dihydroxybenzoic acid

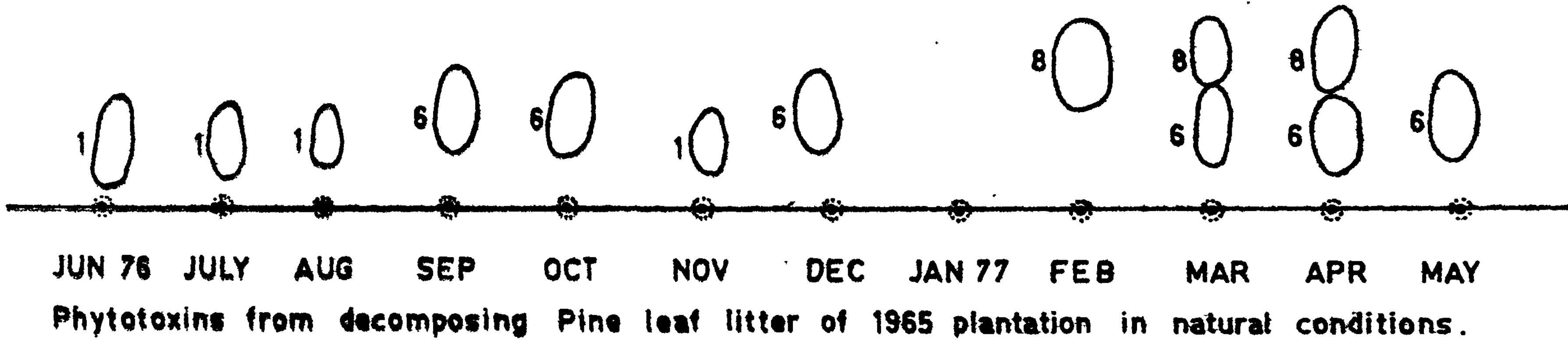
2 = p, coumaric acid

6 = Protocatechuic acid

7 = Gentisic acid

8 = Caffeic acid.





Phytotoxins from decomposing Pine leaf litter of 1965 plantation in natural conditions.

Figure 41

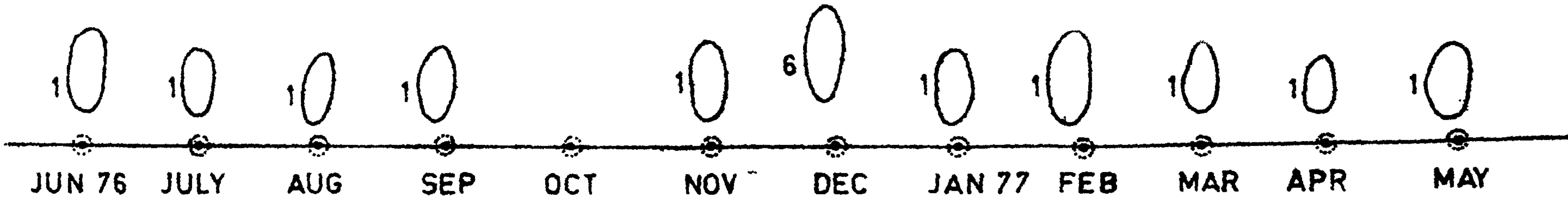
Figure - 42 : Chromatogram showing the presence of various phytotoxins in leachates of decomposing pine litter.

<u>Phytotoxins</u>:

1 = 3,4 dihydroxybenzoic acid

2 = P, coumaric acid

6 = Protocatechuic acid.



Phytotoxins from decomposing Pine leaf litter of 1970 plantation in natural conditions

Figure 42

Figure - 43 : Chromatogram showing the presence of various phytotoxins in leachates of decomposing pine litter.

Phytotoxins :

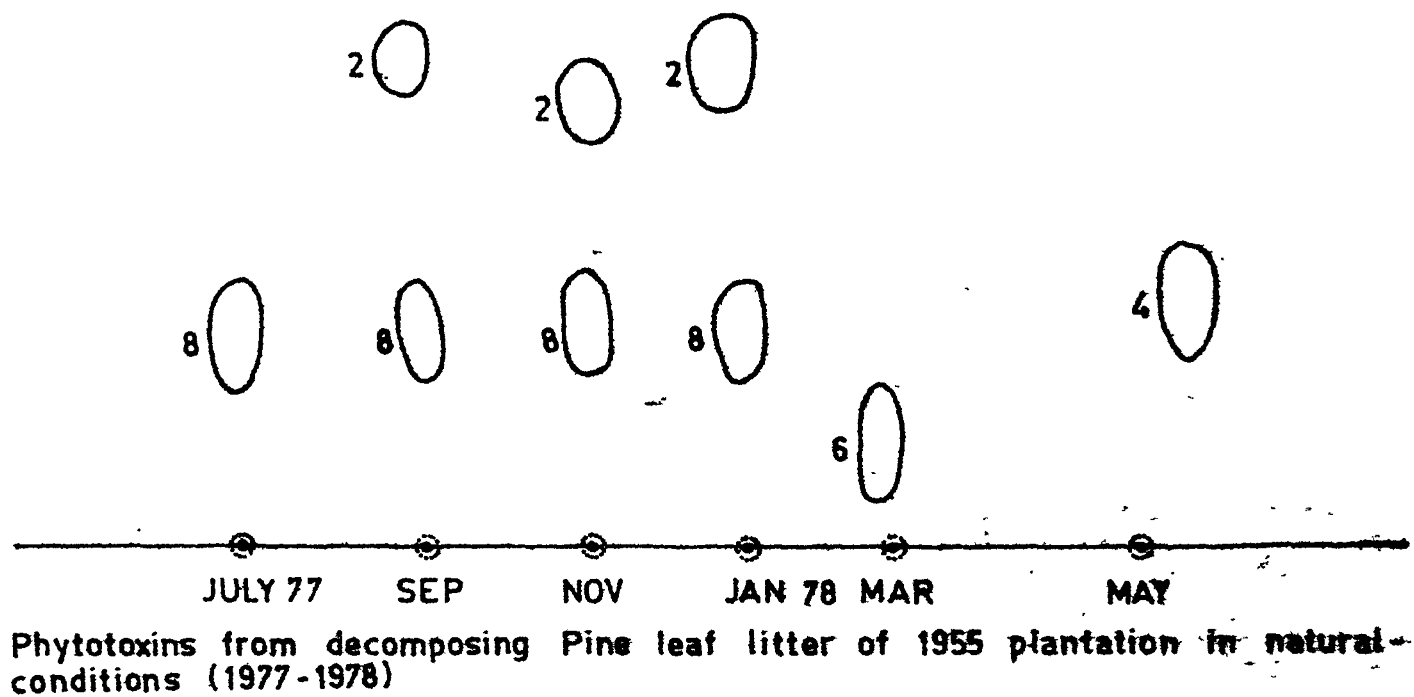
2 = P, coumaric acid

4 = Venillic acid

5 - Quinic acid

6 = Protocatechuic acid

8 = Caffeic acid.



Phytotoxins from decomposing Pine leaf litter of 1955 plantation in natural conditions (1977-1978)

Figure 43

Figure - 44 : Chromatogram showing the presence of various phytotoxins in leachates of decomposing pine litter.

2 = P, coumaric acid

4 = Venillic acid

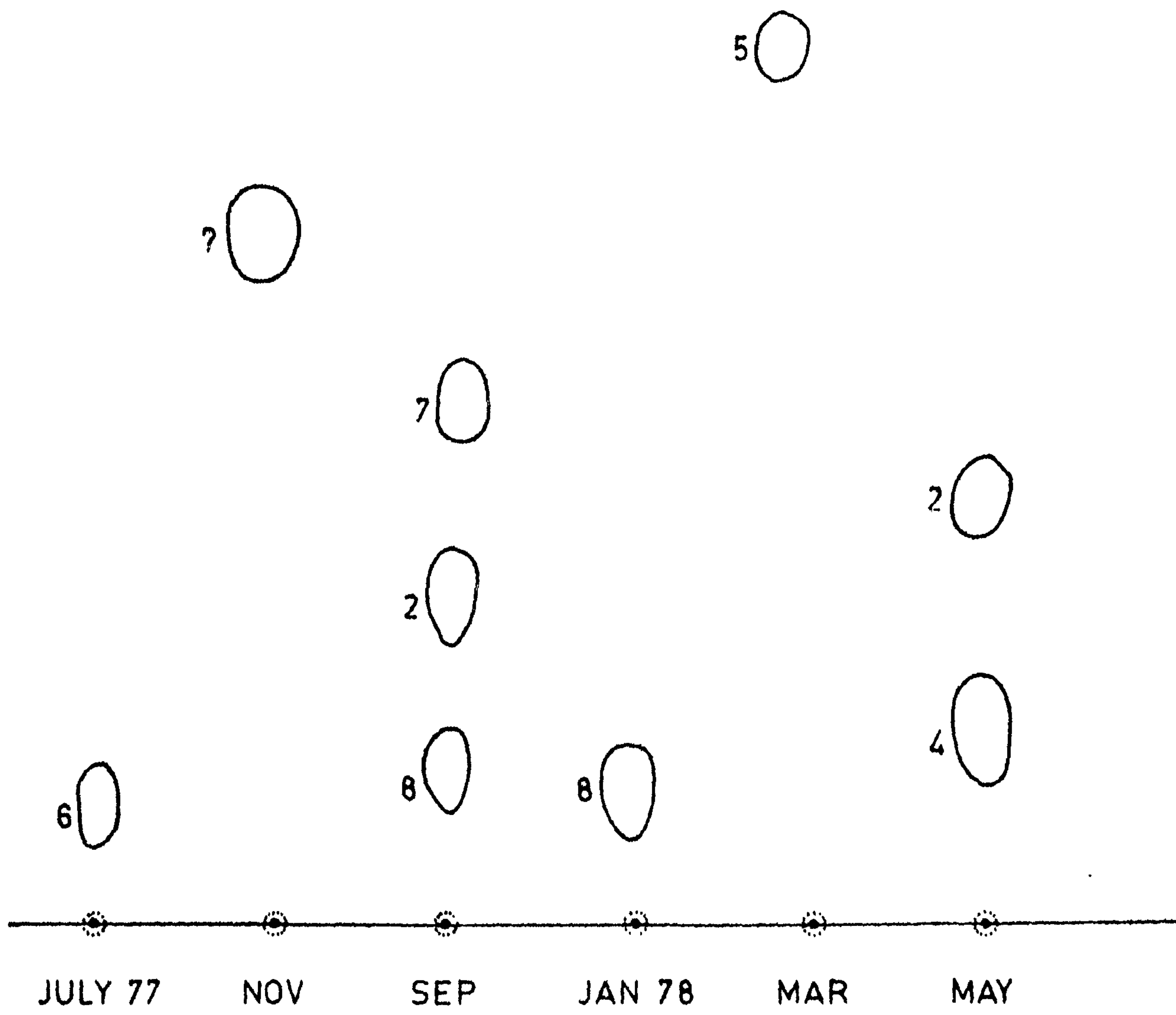
5 = Quinic acid

6 = Protocatechuic acid

7 = Gentisik acid

8 = Caffeic acid

? = Unidentified Rf = 0.600.



Phytotoxins from decomposing Pine leaf litter of 1970 plantation in natural condition (1977-1978)

Figure 44

Figure - 45 : Chromatogram showing the presence of various phytotoxins in leachates of pine litter decomposed in laboratory condition by certain selected fungal species.

Phytotoxins : 1 - 3,4 dihydroxybenzoic acid

2 = P, coumaric acid

5 = Quinic acid

6 = Protocatechuic acid

7 = Gentisic acid

8 = Caffeic acid

? = Unidentified Rf = 0.913.

Selected fungal species - CC = Control

MU = *Mucor hiemalis*

AB = *Absidia cylindrospora*

TR = *Trichoderma viride*

CL = *Cladosporium herbarum*

PE = *Penicillium chrysogenum*

SP = *Sporobolomyces roseus*

MIX = Mixed inoculum.

CO MU AB TR CL PE SP MIX

Phytotoxins from Pine leaf litter after 30 days of decomposition by certain selected fungal sp.

Figure 45

Figure - 46 : Chromatogram showing the presence of various phytotoxins in leachates of pine litter decomposed in laboratory condition by certain selected fungal species.

Phytotoxins : 1 = 3,4 dihydroxybenzoic acid

3 = Ferulic acid

4 = Vanillic acid

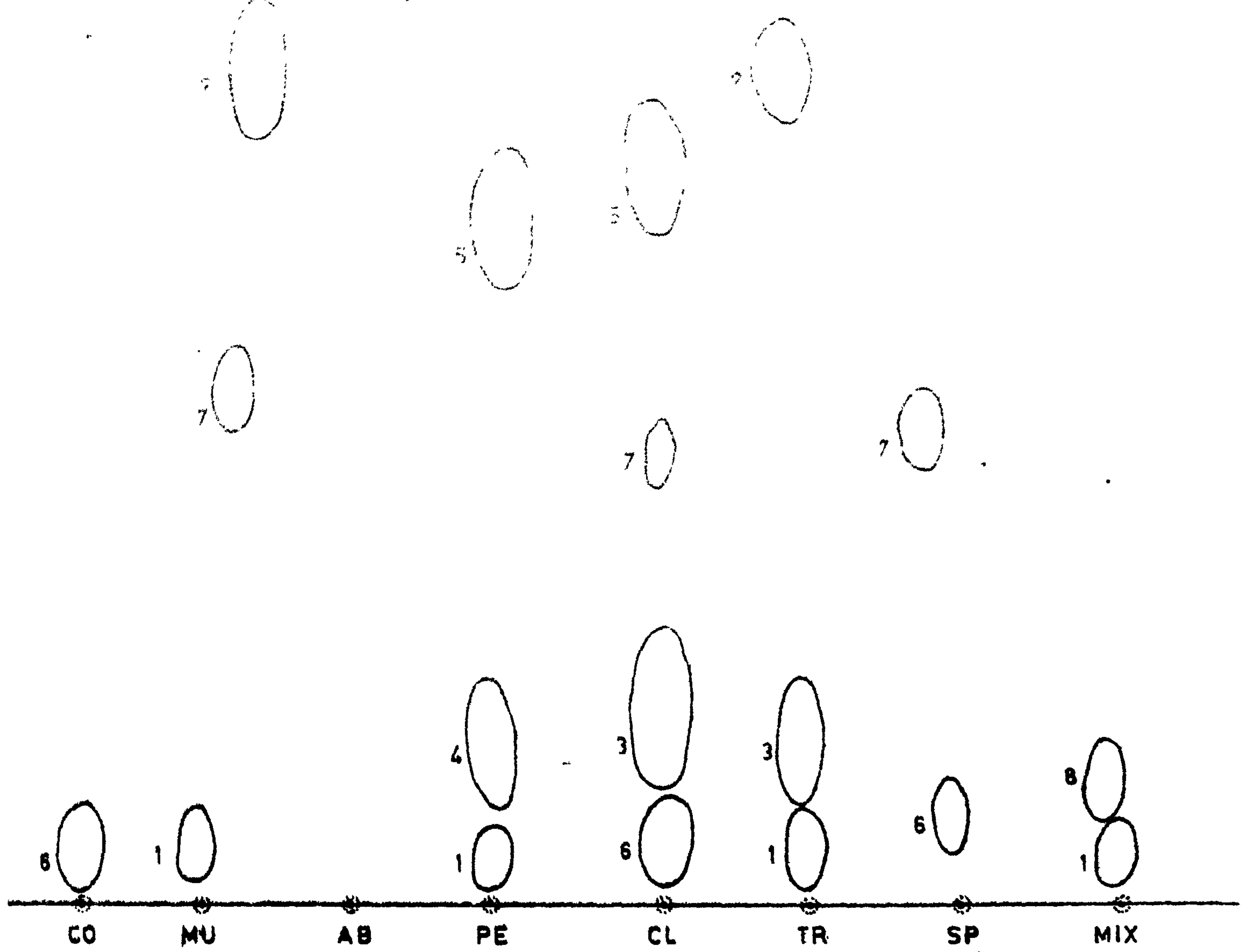
5 = Quinic acid

6 = Protocatechuic acid

7 = Gentisic acid

8 = Caffeic acid

? = unidentified Rf = 0.841.



Phytotoxins from Pine leaf litter after 60 days of decomposition by certain selected fungal sp.

Figure 46

Figure - 47 : Chromatogram showing the presence of various phytotoxins in leachates of pine litter decomposed in laboratory condition by certain selected fungal species.

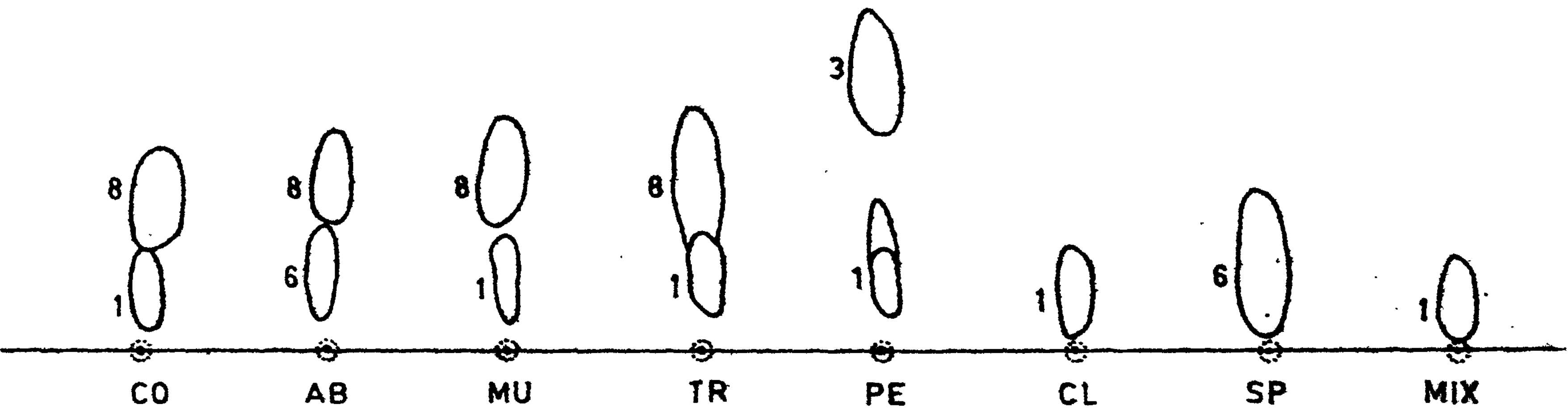
<u>Phytotoxins</u> :

1 = 3,4 dihydroxybenzoic acid

3 = Ferulic acid

6 = Protocatechuic acid

8 = Caffeic acid.



Phytotoxins from Pine leaf litter after 90 days decomposition by certain fungal species.

Figure 47

Table - 60. Analysis of variance for fungal population in decomposing pine litter under different plantations during 1976-1977.

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value 5%	Table 'F' value 1%	Remarks
Between plantations	2	0.94	0.47	0.345	3.40	5.61	Not significant
Between sampling periods	12	111.36	9.28	6.82	2.18	3.03	Significant at both 5% and 1% of level of probabilities
Error	24	32.85	1.36				
Total	38						

The above table reveals that the variation in the fungal population of decomposing pine litter between different plantations is statistically not significant but it varies significantly between different sampling periods.

Table - 61. Analysis of variance for Bacterial population in decomposing pine litter under different plantations during 1976-1977.

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value		Remarks
					5%	1%	
Between plantations	2	334.02	167.01	1.67	3.40	5.61	Not significant
Between sampling periods.	12	3383.47	281.95	2.83	2.18	3.03	Significant at 5% level of probabilities
Error	24	2389.04	99.54				
Total	38						

The above table reveals that the variation in the bacterial population of decomposing pine litter between different plantations is statistically not significant but it varies significantly between different sampling periods.

Table - 62. Analysis of variance for Actinomycetes population in decomposing pine litter under different plantations during 1976-1977.

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value 5%	Table 'F' value 1%	Remarks
Between plantations	2	38.98	19.49	4.71	3.40	5.61	Significant at 5% level of probabilities
Between sampling periods	12	955.46	79.62	19.27	2.18	3.03	Significant at both 5% and 1% level of probabilities
Error	24	99.35	4.13				
Total	38						

The above table reveals that the variation in the actinomycetes population of decomposing pine litter between different plantations and between different sampling periods is statistically significant.

Table - 63. Variation of Fungal population in decomposing pine leaf litter of 1970 plantation (Number of fungi/gm dry litter $\times 10^5$).

Year \ Sampling periods	MAY	JUL	SEP	NOV	JAN	MAR	MAY	TOTAL	MEAN	S.D.	Calculated 't' value	Table 't' value at 5 %
1976-1977	0.97	3.93	3.84	1.14	0.97	2.71	5.56	19.12	2.73	3.25	.312*	.600
1977-1978	2.45	8.77	4.64	2.28	6.58	0.93	1.20	26.85	3.83	8.77		

S.D. = Standard deviation from mean

* = Not significant.

Table - 64. Variation of Bacterial population in decomposing pine leaf litter of 1970 plantation (Number of bacteria/gm dry litter x 10^6).

Year \ Sampling periods	MAY	JUL	SEP	NOV	JAN	MAR	MAY	TOTAL	MEAN	S.D.	Calculated 't' value	Table 't' value at 5%
1976 - 1977	12.0	40.80	6.84	1.68	5.77	11.44	14.20	90.73	12.96	171.54	.039*	.600
1977-1978	9.0	21.86	54.34	2.26	11.07	1.77	32.23	132.53	18.93	362.14		

S.D. = Standard deviation from mean.

* = Not significant.

Table - 65. Variation of Actinomycetes population in decomposing pine leaf litter of 1970 plantation (Number of Actinomycetes/gm dry litter x 10^6).

Year \ Sampling periods	MAY	JUL	SEP	NOV	JAN	MAR	MAY	TOTAL	MEAN	S.D.	Calculated value	Table 't' value at 5%
1976-1977	1.5	5.50	14.48	1.30	1.35	5.94	7.36	30.07	4.29	34.39	.266*	.600
1977-1978	2.4	13.17	26.80	10.17	11.21	7.69	7.00	78.44	11.20	59.43		

S.D. = Standard deviation from mean.

* = Not significant.

Table - 66. Percentage relative abundance and frequency of fungal species on pine leaf litter of 1955 plantation of various periods of decomposition under natural condition (May 76 - May 77).

(Based on dilution plate method)

Sampling periods / Fungal species isolated	PERCENTAGE RELATIVE ABUNDANCE													Frequency
	*MAY76	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY77	
Absidia cylindrospora	-	-	-	-	-	-	-	2.0	-	-	10.0	-	-	R
Circinella sp	-	-	-	-	-	1.0	-	-	-	-	-	-	-	R
Mucor hiemalis	-	-	-	95.0	-	-	1.0	-	-	-	-	-	-	R
Pythium sp	-	12.0	85.0	5.0	-	32.0	22.0	-	-	-	-	-	-	O
Unidentified ascomycetes ?	-	-	-	-	81.0	-	23.0	-	-	-	-	-	-	R
Trichoderma viride	20.0	65.0	13.0	-	1.0	67.0	8.0	25.0	70.0	25.0	-	40.0	40.0	D
Aspergillus flavus	-	-	-	-	-	-	1.0	-	-	-	-	-	-	R
Penicillium chrysogenum	15.0	1.0	-	-	5.0	-	25.0	57.0	-	40.0	40.0	-	-	F
Verticillium sp	-	-	-	-	7.0	-	-	-	-	-	-	-	-	R
Cladosporium herbarum	20.0	-	2.0	-	-	-	-	16.0	10.0	10.0	-	20.0	30.0	F
Sterile white mycelia	40.0	22.0	-	-	-	-	20.0	-	20.0	25.0	20.0	40.0	30.0	C
Sterile brown mycelia	-	-	-	-	3.0	-	-	-	-	-	-	-	-	R
Sterile septate black mycelia	-	-	-	-	3.0	-	-	-	-	-	-	-	-	R
Aureobasidium pullulans	5.0	-	-	-	-	-	-	-	-	-	30.0	-	-	R

*Fungi isolated from fresh leaf litter. D = Dominant (81-100% frequency)
C = Common (61-80% frequency) F = Frequent (41-60% frequency)
O = Occasional (21-40% frequency); R = (1-20% frequency).

Table - 67. Percentage relative abundance and frequency of fungal species on pine leaf litter of 1965 plantation at various periods of decomposition under natural condition (May 76 - May 77).
(Based on dilution plate method).

Sampling periods / Fungal species isolated	PERCENTAGE RELATIVE ABUNDANCE													Frequency.
	* MAY76	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY 77	
Absidia cylindrospora	-	-	-	-	-	-	-	10.0	8.0	-	1.0	-	-	O
Mucor hiemalis	-	1.0	1.0	43.0	-	-	1.0	-	-	-	-	17.0	-	O
Pythium sp	-	13.0	-	-	21.0	20.0	10.0	-	8.0	3.0	-	5.0	-	F
Geotrichum sp	-	-	-	36.0	-	-	-	-	-	-	-	-	-	R
Trichoderma viride	-	86.0	1.0	11.0	60.0	78.0	-	90.0	-	12.0	-	18.0	-	C
Penicillium chrysogenum	5	-	3.0	-	12.0	2.0	42.0	-	31.0	68.0	50.0	-	9.0	C
Verticillium sp	-	-	-	-	-	-	-	-	14.0	-	-	-	-	R
Cladosporium herbarum	30.0	-	94.0	-	-	-	22.0	-	14.0	-	-	29.0	-	O
Fusarium sp	-	-	-	-	-	-	-	-	25.0	-	-	2.0	[illegible]	R
Fusicladium sp	-	-	-	-	-	-	-	-	-	-	-	-	89.0	R
Sterile white mycelia	50.0	-	1.0	-	7.0	-	25.0	-	-	17.0	12.0	1.0	2.0	G
Aureobasidium pullulans	15.0	-	-	-	-	-	-	-	-	-	37.0	-	-	R
Sporobolomyces roseus	[illegible]	-	-	-	-	-	-	-	-	-	-	28.0	-	R

*Fungi isolated from fresh leaf litter.

Table - 68. Percentage relative abundance and frequency of fungal species on pine leaf litter of 1970 plantation at various periods of decomposition under natural condition (May 76 - May 77).
(Based on dilution plate method).

Fungal species isolated \ Sampling periods	PERCENTAGE RELATIVE ABUNDANCE													Frequency
	*MAY76	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY 77	
Absidia cylindrospora	-	-	-	-	-	1.0	-	-	2.0	-	-	-	-	R
Mucor hiemalis	-	-	1.0	83.0	-	1.0	-	-	-	-	-	4.0	-	O
Pythium sp	-	-	99.0	-	8.0	23.0	16.0	67.0	-	-	-	-	-	F
Trichoderma viride	-	21.0	-	14.0	25.0	46.0	6.0	-	-	-	-	17.0	50.0	F
Penicillium chrysogenum	40.0	-	-	-	9.0	5.0	44.0	33.0	12.0	42.0	23.0	1.0	-	C
Verticillium sp	-	39.0	-	-	2.0	-	-	-	18.0	-	-	-	-	O
Cladosporium herbarum	20.0	-	-	3.0	-	-	34.0	-	10.0	-	4.0	8.0	20.0	F
Curvularia sp	-	-	-	-	-	-	-	-	-	2.0	-	-	-	R
Stemphylium sp	-	40.0	-	-	-	-	-	-	-	-	-	-	-	R
Fusarium sp	-	-	-	-	-	-	-	-	31.0	8.0	-	1.0	-	O
Sterile white mycelia	35.0	-	-	-	34.0	24.0	-	-	-	13.0	-	39.0	30.0	F
Sterile black mycelia	-	-	-	-	22.0	-	-	-	-	35.0	-	-	-	R
Sterile pink mycelia	-	-	-	-	-	-	-	-	-	-	-	30.0	-	R
Aureobasidium pullulans	5.0	-	-	-	-	-	-	-	-	-	73.0	-	-	R

*Fungi isolated from fresh leaf litter.

Table 69. Percentage relative abundance and frequency of fungal species on pine leaf litter of 1970 plantation at various periods of decomposition under natural condition (May 1977-May 1978). (Based on dilution plate method)

Sampling periods / Fungal species isolated	PERCENTAGE RELATIVE ABUNDANCE							Frequency
	*MAY77	JUL	SEP	NOV	JAN	MAR	MAY 78	
Mucor hiemalis	-	-	-	-	-	1.0	1.0	O
Pythium sp	-	14.0	-	-	-	-	4.0	O
Phoma humicola	-	1.0	-	2.0	14.0	-	10.0	F
Trichoderma viride	-	11.0	1.0	-	-	1.0	4.0	F
Penicillium chrysogenum	10.0	73.0	40.0	4.0	5.0	11.0	-	D
Cladosporium herbarum	25.0	-	1.0	-	-	-	-	O
Fusarium sporotrichoides	-	1.0	58.0	-	-	-	-	O
Sterile white mycelia	15.0	-	-	2.0	-	-	-	O
Sporobolomyces roseus	50.0	-	-	92.0	81.0	87.0	80.0	C

*Fungi isolated from fresh leaf litter.

Table - 70. Analysis of variance for the original dry weight of pine leaf (*Pinus kesiya* Royle) remaining after various periods of decomposition during 1976-1977.

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value		Remarks
					5%	1%	
Between plantations	2	128.04	64.02	7.05	3.44	5.72	Significant at both 5% and 1% level of probabilities
Between sampling periods	11	1327.74	120.70	13.30	2.40 - 2.23	3.45- 3.12	Significant at both 5% and 1% level of probabilities.
Error	22	199.54	9.07				
Total	35						

The above table reveals that the loss in dry weight of pine leaf litter during decomposition process varies significantly within three different plantations and during different seasons of the year.

Table - 71. Analysis of variance for the original dry weight of Pine leaf (*Pinus kesiya* Royle) remaining after various periods of decomposition during 1977-78.

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value		Remarks
					5%	1%	
Between plantations	1	53.05	53.05	0.548	6.61	16.26	Not significant
Between sampling periods	5	1203.52	240.70	2.487	5.05	10.97	Not significant
Error	5	483.79	96.75				
Total	11						

The above table reveals that the variation in the loss of dryweight of pine leaf litter during decomposition process between plantations and between sampling periods is statistically not significant.

Table - 72. Analysis of variance for moisture content of pine litter during different periods of decomposition under different plantations during 1976-1977.

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value		Remarks
					5%	1%	
Between plantations	2	163.63	81.81	3.28	3.40	5.61	Not significant
Between sampling periods	12	23513.69	1959.47	78.63	2.18	3.03	Significant at both 5% level of probabilities
Error	24	598.11	24.92				
Total	38						

The above table reveals that the variation in the moisture content of decomposing pine litter between different plantations is statistically not significant, but it varies significantly between different sampling periods.

Table - 73. Analysis of variance for pH of decomposing pine litter under different plantations during 1976-1977.

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value		Remarks
					5%	1%	
Between plantations	2	340	0.170	2.69	3.40	5.61	Not significant
Between sampling periods	12	9.57	0.797	12.65	2.18	3.03	Significant at both 5% and 1% level of probabilities
Error	24	1.52	0.063				
Total	38						

The above table reveals that the variation in the pH of decomposing pine litter between different plantations is statistically not significant but it varies significantly between different sampling periods.

Table-74. Course of change of Moisture content (%) of decomposing pine litter of 1970 plantation in two different years (1976-1977-1978).

Sampling periods / Year	MAY	JUL	SEP	NOV	JAN	MAR	MAY	TOTAL	MEAN	S.D.	CALCULATED 't' VALUE	TABLE 't' VALUE AT 5%
1976-1977	8.0	80.0	56.0	5.0	15.0	35.0	70.5	269.50	38.50	944.91	0.036*	0.600
1977-1978	7.5	75.3	80.2	66.4	67.0	27.7	59.3	383.40	54.77	723.64		

S.D. = Standard deviation from mean.

* Not significant.

Table - 75. Course of change in pH of decomposing pine litter of 1970 plantation in two different years (1976-1977-1978).

Year \ Sampling periods	MAY	JUL	SEP	NOV	JAN	MAR	MAY	TOTAL	MEAN	S.D.	CALCULATED 't' VALUE	TABLE 't' VALUE AT 5%
1976-1977	5.45	5.85	6.45	5.20	4.55	5.80	6.05	39.35	5.62	.408	2.11*	600
1977-1978	5.85	7.04	7.00	5.41	6.25	5.90	5.48	42.93	6.13	.488		

S.D. = Standard deviation from mean

* Significant at 5% level.

Table - 76. Analysis of variance for the percentage original dry weight of pine litter remaining after various periods of decomposition by certain selected fungal species under laboratory condition.

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value	
					5%	1%
Between treatments	7	383.23	54.74	4.30**	2.29	3.21
Between sampling periods	5	1018.33	103.66	16.02**	2.48	3.61
Error	35	445.02	12.71			
Total	47					

** Significant at both 5% and 1% level of probabilities.

Table - 77. Analysis of variance for percentage of cellulose in pine leaf litter remaining after various periods of decomposition by certaine selected fungal species under laboratory condition.

Sources of variation	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value	
					5%	1%
Between treatments	7	963.47	137.64	19.19**	2.29	3.21
Between sampling periods	5	2950.96	590.19	82.31**	2.49	3.61
Error	35	251.17	7.17			
Total	47					

**Significant at both 5% and 1% level of probabilities.

Table - 78. Analysis of variance for percentage of hemicellulose present in pine litter after various periods of decomposition by certaine selected fungal species under laboratory conditions.

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value	
					5%	1%
Between treatments	7	91.74	13.11	3.98**	2.29	3.21
Between sampling periods	5	126.83	25.37	7.71**	2.49	3.61
Error	35	115.0	3.29			
Total	47					

**indicates significant at both 5% and 1% level of probabilities.

Table 79. Analysis of variance for percentage of lignin present in pine litter after various periods of decomposition by certaine selected fungal species under laboratory conditions.

Sources of variations	D.F.	S,S.	M.S.S.	Variance ratio calculted	Table 'F' value	
					5%	1%
Between treatments	7	39.03	5.58	1.54	2.29	3.21
Between sampling periods	5	628.69	125.69	34.69**	2.49	3.61
Error	35	127.12	3.63			
Total	47					

**indicates significant at 5% and 1% level of probabilities

Table - 80. Analysis of variance for Co_2 evolution from pine litter at various periods of decomposition by certaine selected fungal species under laboratory condition.

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value	
					5%	1%
Between treatments	7	0.383	0.055	11.0**	2.24	3.10
Between sampling periods	6	0.549	0.092	18.4**	2.33	3.27
Error	42	0.228	0.005			
Total	55					

** indicates significant at both 5% and 1% level of probabilities.

Table - 81. Mean and standard deviation of percentage original dry weight, percentage cellulose, percentage hemicellulose, percentage lignin, remaining in pine leaf litter at different stages of decomposition and evolution of CO_2 in ml hr^{-1} from decomposing litter inoculated with different fungal species under laboratory condition.

Fungal species inoculated	Percentage of original dry weight of litter		%cellulose present		%hemicellulose present		%Lignin present		CO_2 evolution in ml hr^{-1}	
	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
Control	93.04	2.15	41.86	4.98	10.62	1.65	21.27	5.30	0.245	0.158
Absidia cylindrospora	86.25	6.24	30.67	10.37	6.97*	1.82	21.97	3.41	0.447	0.100
Mucor hiemalis	82.11**	5.27	26.47*	8.88	7.97	3.46	20.57	4.27	0.457	0.141
Trichoderma viride	85.50*	6.47	30,23	8.49	6.53*	2.14	19.03	4.67	0.466**	0.084
Penicillium chrysogenum	88.71	5,60	29.77	10.81	5.87**	1.85	19.77	3.77	0.493**	0.122
Cladosporium herbarum	83.61*	7.06	28.67*	6.74	8.20	2.87	19.73	3.86	0.510**	0.130
Sporobolomyces roseus	87.53	7.62	28.50*	10.87	8.07	2.83	19.63	4.03	0.356**	0.130
Mixed	85.74*	4.90	27.83*	8.77	6.63*	2.42	20.00	5.09	0.382**	0.134

S.D. = Standard deviation; ** = indicates significant at 1% level of probabilities from control.

* = indicates significant at 5% level of probabilities from control.

Table - 82. Course of change of pH of pine leaf litter at various stages of decomposition by certain selected fungal species under laboratory condition.

Fungal species inoculated / Periods of decomposition	MAY 78	JUN	JUL	SEP	NOV	JAN 79
Control	5.26	5.75	5.05	5.50	5.00	4.95
Absidia cylindrospora	5.57	5.59	5.51	5.65	5.00	4.99
Mucor hiemalis	6.01	5.43	5.56	5.66	5.20	5.00
Trichoderma viride	5.43	5.70	5.90	6.00	5.25	4.85
Penicillium chrysogenum	5.46	5.50	5.56	5.70	5.00	4.92
Cladosporium herbarum	5.46	5.63	4.63	5.50	5.15	4.95
Sporobolomyces roseus	5.50	4.82	5.44	5.45	5.10	4.90
Mixed	5.99	5.71	5.30	5.37	5.00	4.82

DISCUSSION

A. <u>Field decomposition studies</u> :-

Initially lesser microbial population from litter kept on the forest floors may be partly because of resistant nature of pine needles towards the first wave of microbial attack (Figs. 21-24). Further, low moisture content at the time (Figs. 21-24) perhaps did not favour leaching of soluble nutrients to support the microbial growth. As the litter within the bags were exposed further to weathering on the forest floor, more soluble nutrients were available in the form of soluble sugars and amino acids as these two constituents were rapidly lost at the early stages of decomposition (Figs. 35,36). Moisture content of litter which was fairly high due to high rainfall, also probably helped in the built up of the microbial population (Figs.21-24). It seems, therefore,that moisture content and nutrient status of litter under natural condition play a significant role in the growth of microflora during the process of decomposition. Rai (1973) stressed the importance of moisture content and nutrient status of substrate in the succession of fungi on decaying leaves. Jensen (1974) also emphasized that in more resistant litter, development of bacterial flora is slower, and the number may increase gradually over a long period owing to improved moisture conditions.

Again an excess moisture content led to a decrease in microbial-population on decomposing pine litter (Figs.21-24). This could be due to lack of aeration which affected microbial activity adversely (Mikola, 1954;

Witkamp, 1966).

Significant seasonal changes in the microbial population on decomposing litter was observed. (Tables 60,61,62). Seasonal changes in the atmospheric temperature, rainfall and humidity (Fig.13) seems to have pronounced impact on the competitive saprophytic ability of microbes, and on the quantity of nutrients present in decomposing litter. Holm and Jensen (1972) also observed that the bacterial numbers in decomposing litter of _Fagus sylvatica_ were affected more by the changing weather conditions than by age of the litter.

A gradual increase in the litter microflora at the early stage of decomposition (Figs. 21-24) may be also explained due to intensive activity of the fauna which exposed litter surface for rapid decomposition. This has been confirmed by the studies conducted on the litter under the same conditions by Reddy (unpublished). The importance of the micro fauna in the litter decomposition has been well documented by several workers,(Abrahamsen, 1972; Anderson,1975)

The increase in the microflora during rainy (July-September) and summer (March-May) and subsequent decrease in the population during winter months shows a definite affect of weathering on changing microflora of decomposing litter under natural condition (Figs. 21-24).

pH of pine litter invariably affected the growth

of fungi of the decomposing litter. Acidic nature of litter perhaps favoured the growth of more and more fungi on decomposing litter. The role of bacteria in decomposition of acidic litter is not clear (Miller, 1974). However, in the present study a overwhelming dominance of bacteria and actinomycetes on the decomposing litter suggests that pH is not a critical factor for the growth of these organisms in the nature where other factors are also operating simultaneously. Mikola et al (1957) also reported a dominance of bacteria on Pinus sylvestris litter in Finland. Stout (1961) and Goodfellow et al, (1968) on the otherhand reported a fairly large quantity of bacteria in litter of Pinus radiata and Pinus nigra respectively.

Insignificant variation in the fungal and bacterial population of decomposing litter between different pine stands is in agreement with the result of Witkamp (1966)(Tables 60,61). It may partly be due to the exposure of litter practically under the influence of same environment of the locality and also due to the presence of almost similar nutrient status of litter of all the stands. The quantity of cellulose, hemicellulose, lignin, total sugar and aminoacids are almost same in the litter of all the stands (Figs.29, 33,35,36).

A small number of fungi including few phycomycetes, yeasts and majority of deuteromycetes particularly species of Trichoderma, Penicillium, Cladosporium, appeared on the decomposing needles of

all the plantations without any regular sequence (Tables 66-69). Several of them particularly *Pythium* sp., *Penicillium chrysogenum*, *Trichoderma viride*, *Cladosporium herbarum* and sterile white mycelia were isolated with high frequency but majority of the fungi exhibited low percentage occurence (Figs.25-28). Many of them were present on the litter with fairly large population on different sampling periods. The dynamic changes in the microbial balance (Garrett, 1963), certain antagonistic factors (Lockwood, 1964), nutrient status of the decomposing litter and the release of different substances seems to govern the discontinuous occurence of litter fungi in varying frequencies.

The reason for the occurence of very small number of fungal species on decomposing pine litter in comparison to the litter of other tree species may be due to release of phenolic compounds (Phytotoxic substances) which have been identified from decomposing litter (Figs. 40-44) (Tables 66-69). In a few cases, however, phenolic substances particularly Ferulic acid is found to be utilized by microfungi of both litter and soil (Black and Dix, 1976). The inhibitory action of other phytotoxins in litter decomposition is well documented by Patrick *et al* (1964); Toussoun *et al* (1968); Patrick, (1971) and Lockwood,(1963).

Also 'serial dilution plate technique' for the isolation of fungi from litter might have favoured the

growth of only those organisms which are nutritionally and competitively better favoured on the plates. Inspite of this Lacunae, this method has been widely used for such studies (Guillemat et al, 1957; Witkamp 1963). Failure to interpret plate counts in the past is presumably due to the results based on too few samplings. Dilution plate method therefore may not be a reason for low counts of the fungi. Production of phytotoxins seems to play a dominant role for such a low number of fungal species on decomposing litter. Mishra et al (unpublished) recently observed the inhibition in the growth of certain fungi in pine litter leachates which they expect to be due to the production of some phytotoxins from pine needles.

The occurence of two phycomycetes, Pythium sp. and Mucor hiemalis as dominant forms at the early stage of litter decomposition is due to their greater affinity towards sugars and aminoacids liberated (Figs. 25-27). In general early colonizers of conifers litter are bacteria, ascomycetes, deuteromycetes and some basidiomycetes, which attack simple carbohydrates and cellulose. These are followed by phycomycetes, particularly members of mucorales which can utilize the fungal breakdown products, perhaps in combination with meiofauna (Miller, 1974). Considerable decrease and rather disappearance of phycomycetes at later stages may be due to changed nature of the substrate.

The present study showed that majority of the

total mycoflora is represented by the deuteromycetes, particularly Trichoderma viride, Penicillium chrysogenum and Cladosporium herbarum (Figs.25-28). The deuteromycetes have been found to play the most important role in pine litter decomposition. Occurence of the above mentioned fungi on decomposing litter could be explained in terms of their adaptation to utilize different phytotoxic compounds from decomposing litter (Black et al 1976). The nature and the environmental conditions of the microhabitat exercise a broad selective effect upon the kind of microorganisms that enter into the competition. A definite correlation appears between the substrate and the different fungi that colonize it. As plant materials are decomposed by fungi, a variety of organic substances are presented in turn to a succession of ecological groups of fungi, each organism or group of organisms altering the organic constituents until complete decomposition occurs (Alexander, 1961; Garrett,1951). These organic constituents serve the basis for classfying the fungi into different ecological groups; the sugar fungi (Burges, 1939) that decompose simple carbon compounds and sugars, mostly the phycomycetes which are called 'primary colonizers'. These, in turn, are followed by cellulose decomposing fungi which include the members of ascomycetes and several imperfect fungi. These forms are grouped as 'secondary colonizers'. The fungi which are responsible for the decomposition of lignin like

complex forms of nutrients, appear in the last, and most of the basidiomycetes fall in this category (Garrett, 1963).

The presence of greater number of sterile forms in high frequency throughout decomposition period may be due to their high saprophytic ability which facilitate their survival even under adverse circumstances (Figs.25-27). Similar views were put forward by other workers as well as (Nicot, 1960; Gadgil,1965; Mishra, 1966 a,b; Singh, 1967; Kanaujia, 1973).

The rate of decomposition of Pine litter is extremely slow (Figs.29-30). It is perhaps primarily due to a combination of a low-base status substrate (Fig.31) containing resistant substances such as waxes, resins and lignin and the cold climate. These conditions do not favour an active microflora or meiofauna (Tables 66-69). Miller (1974) also put forward a similar view regarding the decomposition of coniferous leaf litter. Kendrick (1959) observed that a pine needle remains in the L layer for about 6 months, in the F layer for 2 years and in the F_2 layer for 7.5 years. Guittet (1967) also made a similar estimate for the rate of pine litter decomposition in France, the time to reach pine litter into humus stage being approximately 10 years. Mayer (1962) estimated that duration for the decomposition of Picea abies varied between 17 and 31 years for complete mineralization. The loss in dry weight of Pine litter at the early stage of decomposition

i.e. from June-September was little faster than rest of the study periods (Figs.29-30).This is primarily due to an increase in the moisture condition of the litter high rainfall and increase in atmospheric temperature (Figs.13 and 31).These environmental factors have been suggested to govern the rate of litter decomposition (Williams and Gray 1974). Also during that periods litter microflora increased very rapidly probably due to rapid utilization of soluble sugar and amono acid from Pine litter (Figs.35 A,B and 36 A,B).

Again an excess of moisture in litter (Fig.31) probably suppressed the rate of decomposition during the middle and latter stages due to lack of aeration and reduction in microbial activity (Mikola, 1954; Witkamp, 1966). It has been noted that soil moisture and temperature control the movement and feeding activity of microfauna, (Hayes, 1965; Metz,1971; Usher,1970). Exclusion of larger soil fauna including earthworms due to small mesh size of the litter bags used in the present study may also account for the slow rate of decomposition.

There have been few detail studies specifically on the chemistry of the decomposition of coniferous litter. Sokolov and Karpova (1965) studied decomposition, humification and mineralization in a mixed pine forest podsol (Miller,1974). They found that first substances decomposed were the water soluble, alcohol and benzene extractives such as starch, hemicellulose and

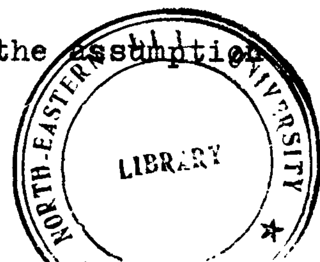
amino acids, Lignin was decomposed later. Almost a similar result was observed in the present investigation where rate of disappearance of total sugar, amino acid and hemicellulose was much faster than any other components (Figs.35 A, 35 B, and 36 A and 36 B). These studies suggest that autolytic process in decomposing litter lead very quickly to the decomposition of sugars. Proteins and amino acids and the compounds which are difficult to hydrolyse are lignin and cellulose. As the population of deuteromycetes fungi increased during decomposition more cellulose was decomposed (Fig.33-34). The constancy of the percentage of lignin in decomposing litter under natural condition could be due to the slow exposure of this constituent to microbial metabolism through the removal of other components.

A substantial increase in the concentration of total sugar and amino acids during the early stage of litter decomposition (Figs. 35 A and 36 B) suggests the possibility of the incorporation of fungal mycelia and small animals with litter due to the increased activity of microbes during this period. Such increase in the absolute amount of nitrogen in the oak litter during first 10 months of decomposition was observed by Gilbert et al (1960) and he correlated this with presence of small animals invading the litter, which could not be sorted out during estimation. Earlier, Saito (1957) also demonstrated a similar increase in nitrogen in beech litter during the course of decomposition but he did not suggest a possible cause. Recently Das, (unpublished) also accounted a similar increase in percentage nitrogen

in pine litter during certain periods of decomposition under natural condition. Litter bags method has become a standard technique for estimating the decomposition rate of leaves, comparing the decomposition of various species, and characterizing different geographic areas (Ando 1970; Gosz et al 1973; Macauley 1975; Mikola 1960; Witkamp, 1966). A significant difference was observed in the rate of pine leaf decomposition under different stands in the same locality (Table 70). It may primarily be due to certain abiotic factors which could not be accounted here. Mikola (1960), for instance, found no clear difference in decomposition of litter in pine and spruce stands in the same locality but he did find a difference between stands in Southern and Northern Finland.

Measurements of Co_2 production in the field are frequently used in estimations of rates of organic matter breakdown (Ross et al 1973). Significant positive correlations between Co_2 evolution and rate of weight loss from the present study will aid in the prediction of decay rates and rates of mineral turnover in the forest floor under various ecological conditions. Witkamp (1966) also observed a similar positive correlation between Co_2 evolution and rate of weight loss of decomposing litter under field conditions.

Significant positive correlations also existed for mean annual Co_2 production and a combined estimate for microbial population ($r = 0.810$ $p \angle 0.001$). This estimate equates all bacterial and fungal counts on the assumption



that under the wide variety of ecological conditions both counts represent equal breakdown potentials (Witkamp,1963).

B. Laboratory decomposition studies :-

A significant difference in the dry weight loss of litter caused by different fungal species under laboratory condition indicates that different fungal species have different decomposing ability even under the identical environmental situations (Table 76)(Fig.37). The present study was carried out at room temperature for all the species tested and the moisture content of soil was also identical for each set. The variation in the percentage loss of cellulose and hemicellulose (Tables 77-78) (Fig.38). by different fungal species suggests that the rate of decomposition of an organic material is closely linked with growth of the organisms associated with decomposition. The rate of decomposition of any substrate is generally proportional to the growth rate of its decomposers (Parnas, 1975).

However, if we compare the rate of pine litter decomposition in laboratory condition with that of forest floor we find that comparatively less amount of litter was decomposed under the laboratory condition. Only 7-25 % of litter was decomposed in nine months (Fig.37). This may be primarily due to the total exclusion of decomposer fauna from both the soil and litter due to sterilization, and also due to several biotic and abiotic factors, which influence the rate of litter decomposition under field conditions The importance of microfauna in decomposition of forest litter has been well documented by several

authors (Heath and King, 1964; Minderman et al, 1967; Abrahamsen, 1972; and Anderson, 1975). Their exclusion from the litter will substantially decrease the rate of decomposition. In laboratory experiments, where faunal activity was excluded, Broadfoot and Pierre (1939) and Lossaint (1953) found that the most important factors controlling the decomposition rate were the contents of water soluble organic matter, nitrogen, excess-base and calcium. The soluble organic substances were important mainly in the initial phase and excess base and soluble calcium during the later stages of decomposition.

Plants contain a variety of polyhydroxy phenols which vary from 5% to 15% of their dry weight. Some may be extracted with water to give tannins and they may be thus leached from litters into the soil. There is increasing amount of evidence for the importance of these substances in controlling rates of litter decomposition (Williams and Gray, 1974). During the course of the present investigation, it was observed that pine leaf litter decomposing in sterile soils amended separately with certain fungal isolates produced more phytotoxic substances during the early stage (Figs. 45-47). Production of excess of these substances affected the growth of the fungus adversely and this probably accounted for slow rate of decomposition at the later stage.

As regards the loss of cellulose, hemicellulose and lignin content of pine litter different species of fungi exhibited varied ability to decompose the three

constituents (Fig.38). The more loss of cellulose and hemicellulose caused by Mucor hiemalis, Trichoderma viride, Cladosporium herbarum, Sporobolomyces roseus, Absidia cylindrospora, Penicillium chrysogenum and mixed inoculum (Table 81), suggests their more affinity towards the rapid utilization of carbohydrates for better adaptation.

In the present study the active degradation of cellulose and hemicellulose by the test fungi probably led to the production of toxins which perhaps adversely affected the growing fungi further to decompose lignin (Figs. 45-47). It is believed that these toxic compounds are intermediate breakdown products of cellulose and other plant constituents (Patrick and Toussoun, 1965). Miller (1955) and Miller et al (1958) have also made the similar reports.

More carbondioxide production from litter inoculated with Trichoderma viride, Penicillium chrysogenum, Cladosporium herbarumk Sporobolomyces roseus and mixed culture suggests their efficiency to decompose the substrate during different periods (Table 81). A substantial increase in the evolution of Co_2 from litter of all the sets during early stages of decomposition indicates initially a rapid breakdown of litter by different fungi (Fig.39). With the progress of decomposition, however, more toxic compounds were perhaps produced due to which the growth of the inoculated fungi was probably inhibited on litter and therefore a decline in the carbon dioxide production was observed at the latter stages (Fig.39).

CHAPTER - 4.

EFFECT OF THE AMENDMENT OF PINE LEAF LITTER ON SOIL MICROBIAL POPULATION.

INTRODUCTION

Since the recognition of the phenomenon of mycostasis in soil, attention has been given to the role of amendments on the growth and activities of soil microbes. The theory underlying the biological control of soil-born plant pathogens depends on amending the soil with crop residues. This activates the saprophytes for active decomposition resulting into a state of microbial competition and antibiosis which are detrimental to the pathogens (Forbes, 1974).

The production by soil microorganisms of chemicals toxic to others is well known and it is frequent during active growth of the microbes. It is untenable to suggest that microorganisms, despite their vast numbers in soil compete for physical space, it is expected, however, that they compete for sites, free from toxic substances. Plant residues added to the soil are thus both a source of available nutrients, and are a fresh physical substratum in which antibiosis is at a minimum.

The addition of plant residues to soil provides soluble and easily available energy materials which results in explosion of microbial activity (Burges, 1967).

Plant debris is usually a principal substrate added to the soil system and it plays an important role in maintaining soil fertility, affecting both the physical structure of the soil and its nutrient status. The study of plant residues and their decomposition has been vigorously pursued with a view to biological control of plant pathogenic organisms.

The decomposition of organic materials of plant and

animal origin is an important stage in the re-circulation of plant nutrients via the soil-plant - animal cycle (Floate 1970). There have been few detailed studies specifically on the chemistry of the decomposition of the coniferous leaf litter. Sokolov and Karpova, (1965) studied decomposition, humification and mineralization in a mixed pine forest podsol. They found that the first substances decomposed were water soluble alcohol and benzene extractives such as starch, hemicellulose and aminoacids. Inorganic residues and lignin were decomposed later. The remaining humus contained humic acid, fulvic acid, various hydrolysate residues and 7% of waxes and resins. It is a well known phenomenon that dead leaves release a variety of inorganic salts and organic substances when soaked in water. Typically 1-3% of the dry weight of the needles may be lost after soaking for one day. Nykvist (1959) stated that it is likely that these leachates influence the growth of soil fungi during the early stages of decomposition.

There are evidences that decomposition of plant residue in the soil results in the formation of compounds which may have either favourable or unfavourable effects on plants as well as on soil microorganisms. The idea developed after the work of Patrick, et al (1964), Toussoun et al (1968). Toussoun et al (1969) demonstrated that when *Fusarium* chlamydospores in forest soil were exposed to pine duff extracts, 95-98% germinated and lysed.

Recently Freddi et al (1975) demonstrated the lysis of germ tube of *Fusarium oxysporum* in non-sterile soil in presence of five acids extracted from pine needles.

They suggested that presence of these acids in pine forest soil may be partly responsible for the absence of _Fusarium_ spp from the soils.

Biological control appears to be a highly, if not the most promising line of research on root diseases. Ordinary soil contains numerous microorganisms antagonistic to root pathogens. However, practically nothing is known of the conditions that determine abundance and activity of these beneficial organisms. The various ways in which they affect pathogens are poorly understood and they are exceedingly difficult to study under field conditions. If organic amendments can be used to promote growth of appropriate antagonistic microorganisms as an effective substitute for chemicals in control of root diseases, many of the undesirable features of chemical treatments may be avoided. Additional benefits may also be obtained in the form of improved soil structure and fertility.

The present investigation was conducted to study the effect of litter on the soil microflora. The effort has also been made to detect the phytotoxins released during the decomposition of litter. The germination behaviour of certain soil fungi in leachates collected from litter and amended and unamended soils has also been investigated. The decomposing litter, a part of the soil system, may be effective in regulating the soil microflora in various ways and this matter has been explored during the present study.

REVIEW OF LITERATURE

The work on effect of organic matter amendments on soil microflora was pioneered by McLean and Wilson (1914) and Waksman and Starkey (1924). Jensen (1931) observed that addition of farmyard manure and fresh straw in acidic soils increased the species of *Cephalosporium*. Singh (1937) showed that number of microorganisms in soil was directly influenced by different manural treatments.

The negative effect of organic amendment on soil fungi has been reported by many workers (Guillemat and Montegut, 1960; Das, 1963; Reddy and Rao, 1965). The effect of organic amendment in soil was studied by Davey and Papavizas (1960). They added immature (green) and mature (dry) plant material to a loamy sand green house soil and Elsinoboro sandy soil and determined their effect on rhizosphere microflora. They observed an increase in fungi by amendments. Qualitatively few genera were stimulated whereas others were unaffected, reduced or apparently eliminated. Toussoun *et al* (1963) studied the effect of phytotoxic decomposition products of various crop residues on the chlamydospores of *Fusarium solani* in soil.

Maurer and Baker (1964, 1965) studied the effect of chitin and lignin amendments in the soil and observed a decrease in *Fusarium* population of bean plants. The effect of chitin amendment on the root fungi of peas was studied by Khalifa (1965). A considerable decrease in the severity of pea wilt caused by *Fusarium oxysporum* was reported by him. He also studied the effect of pectin,

glucose, cellulose and laminarin, and found them less effective than chitin against Fusarium wilt.

The decrease in fungal population due to the amendment of various organic matter has also been reported by Dransfield and McDonald (1966); Snyder (1960); Stover (1962); Henis et al (1967); Singh and Pandey (1966); Adams et al (1968); Lu (1968); Ghaffar et al (1969) and Manning and Crossan (1969).

Huber and Watson (1970) studied the effect of various organic amendments on soil borne plant pathogens. They found that disease severity was correlated with the effect of specific crop residues in soil on nitrification.

Garrett (1970) pointed out that in general the beneficial effects of crop residue decomposition on soil greatly outweigh the harmful effects. Nevertheless, work on phytotoxic decomposition products has been important in helping to unreveal such problems as 'sick soil' associated with replanting fruit trees.

Kanaujia (1973) observed that the harvesting of the crop and various amendments of the soil exerted little effect on the mycofloral succession except that both these factors hastened the decomposition of the plant material. He recorded higher fungal population in majority of the plots amended with organic manure, fertilizers, and plant-parts.

Vishwanath et al (1975) investigated dehydrogenase activity and microbial population in amended and unamended soils with incubation. Johri et al

(1975) also studied the ecology of thermophilic mycoflora of amended and unamended soils with incubation. They found little fluctuations in the population of fungi and actinomycetes during the entire period of incubation, irrespective of whether they received nutrients or not.

Okpala (1975) studied the effect of various organic soil amendments on "damping off" of Lettuce caused by *Corticum praticola.*

Various organic matter amendment in soil and its effects on soil microbial activity was studied by Vertraete and Voets (1976). They revealed a strong effect of organic fertilization on soil microbial activity.

Chavez *et al* (1976) studied the effect of the amendment of crop residues in soil to control *Phytomatotrichum*, root rot of cotton. Mehrotra and Tewari (1976) studied the effect of soil organic amendments on control of foot rot of *Piper betel* caused by *Phytophthora parasitica*.

Grover (1977) studied the effect of various organic and inorganic fertilizers and organic manure on the mycoflora of decomposing plant material in soil. He observed that fungal population of the soil in different sets of amendments exhibited a regular pattern. He also observed that the highest fungal population in soil was associated with the sets amended with organic manure.

MATERIALS AND METHODS

To investigate the effect of amendment of pine leaf litter on soil microflora the experiments were conducted in net house for a period of eight months i.e. from June 1977 to January 1978. Two types of litter : freshly fallen and semidecomposed were used in this experiment. Both type of needles were collected seperately from the oldest age group of pine stand (1955 pine plantation) and were brought to the laboratory. Soil from the same pine stand was also collected and brought to the laboratory.

The experiment was conducted in plastic pots. 1.0 kg. of unsterilized soil was filled in each pot. Freshly fallen needles and semidecomposed needles were amended separately in each pot at the rate of 500 g per pot. The amount of the litter used gave almost the same thickness on surface of the pot soil which is normally available on the forest floor. Three replicate sets were maintained for each amendment and for each sampling period. Likewise three replicate sets were left unamended and kept as control. All the pots were kept in net house under natural condition.

Moisture status of the soil in all the pots was adjusted regularly to field capacity by adding equal quantity of water.

The first sampling of the decomposing litter (freshly fallen needles and semidecomposed needles) and soil was done after one month of amendment and subsequent samplings were also done at an interval of one month.

During each sampling periods soil was collected in sterilized polythene bags separately from the pots amended with freshly fallen pine needles, semidecomposed needles and unamended pots. In each case soil from these replicate pots was mixed thoroughly to minimize local variation. 50 g of soil in each case was transferred aseptically to the 250 ml conical flask containing 100 ml of 70% ethyle alcohol. Flasks were hand shaken vigorously for half an hour keeping the mouth closed with sterilized cotton plugs. These washings were then filtered The filtrate was centrifuged at 3000 rpm for 15 minutes and again filtered through Whatman filter paper No.1. The filtrate was concentrated to 10 ml in electric vacuum oven below 50°C and was kept at 3°C in a refrigerator for the detection of phytotoxins. The filtrate was also used to test the germination ability of the spores of different fungal species.

Likewise litter samples (freshly fallen needles and semidecomposed needles) were also collected during each sampling period from the pots and 50 g of litter from each sample was transferred to 250 ml conical flasks containing 100 ml of 70% ethyle alcohol. The flasks were hand shaken vigorously for 15 minutes and were left overnight. These washings were then filtered. The filtered leachate was centrifuged at 3000 rpm for 15 minutes and again filtered through Whatman filter paper No.1. The leachates were then concentrated to 10 ml in electric vacuum oven below 50°C and were kept at 3°C in a refrigerator for the detection of phytotoxins. The

leachate was also used to test the germination ability of the spores of different fungal species.

<u>Isolation of microflora from amended and unamended soil</u> .- Bacteria, actinomycetes and fungi were isolated from the soil samples during each sampling period. Population of all the three groups of organism was studied to observe the effect of pine litter amendment. Percentage relative abundance and percentage frequency of fungi was calculated as described in the materials and methods (Chapter 1).

a) <u>Isolation of fungi</u> - Warcup's soil plate method was followed for isolation of fungi from the soil samples as described in the materials and methods (Chapter 1).

b) <u>Isolation of bacteria and actinomycetes</u> - Dilution plate method was followed for the isolation of bacteria and actinomycetes throughout the sampling period, as described in the materials and methods (Chapter 1). Bacteria and actinomycetes population was assessed quantitatively only, no attempt was made to identify the species.

<u>Detection of phytotoxins from soil extract and litter leachates</u> : The method for the detection of phytotoxins was followed as described in the materials and methods (Chapter 3).

<u>Spore germination of certain soil fungi in the soil washings and litter leachates</u> : Certain soil fungi usually dominant and commonly occuring in the experimental sites were selected for this experiment. The selected fungal

species were _Absidia cylindrospora_, _Pythium_ sp. _Trichoderma viride_, _Penicillium chrysogenum_, _Verticillium_ sp and _Fusarium sporotrichoides_. The effect of soil washings and litter leachates on the germination of above mentioned fungi was studied in cavity slides by hanging drop method. The spores were incubated at 25°C for 18 hours, and the number of germinated spores was counted at regular interval till the number becomes constant. The percentage of germination was determined in each case on every sampling date.

pH and moisture content of soil - pH and moisture content of amended and unamended soil was determined at each sampling period by electric pH meter and oven dry weight basis.

RESULTS

The results of the experimental findings on Pine litter amendments in soil are represented in the following order -

1) Changes in the microbial population (fungi, bacteria, actinomycetes) and pH of unamended and amended soil are represented in the Figs. 48 and 49 respectively. Statistical analyses of the results are depicted in the Tables 83-86.

2) Records of percentage relative abundance and frequency of fungal species isolated from unamended and amended soils are represented in the Figs 50 and Tables 87-89. Statistical analyses are depicted in the Tables 90-94.

3) Records of percentage germination of spores of certain soil fungi in pine litter leachates are represented in the Fig.51 and analysis of variance of the results are depicted in the Tables 95-100.

4) Records of percentage germination of spores of some fungi in soil washings of unamended and amended soils are represented in the Fig.52 and statistical analyses of the results are depicted in the Tables 101-106.

5) Phytotoxins detected from pine litter and from unamended and amended soils are represented in the Figs.53-54.

1) Changes in the microbial population (fungi, bacteria and actinomycetes) and pH of the soil unamended and amended with pine leaf litter :-

The fungal population of the soil in different sets exhibited almost a regular pattern. It was always maximum in the beginning and minimum in the end (Fig.48 A). It was

observed that the fungal population fairly increased in the soil amended with both fresh and semidecomposed litter, except in the month of January (Fig.48 A). A regular decreasing trend in fungal population in the control set was recorded (Fig.48 A). Inthe amended sets this trend was not observed and the fungal population was almost the same throughout the observation period except in the month of January. The maximum fungal population in unamended sets was recorded in the month of July and October respectively and the minimum population was recorded in the month of January in all the three sets (Fig.48 A). The variation in the fungal population of soil due to litter amendments was not significant statistically. The variation however, due to the effect of season was significant (Table 83).

The bacterial population increased in all the sets during the month of August and subsequently decreased in the amended sets during the later periods (Fig.48 B). The peak population in unamended soil (UN) was however observed in the month of October, while in soil amended with fresh litter (Sl_1) and semidecomposed litter (SL_2) the maximum population was recorded in September and August respectively. A drop in the bacterial population was observed in the soil amended with fresh litter (SL_1) from the month of September to January and soil amended with semidecomposed litter (SL_2) from August to January (Fig.48 B).

Statistically no significant effect of litter

amendment on bacterial population was observed. The variation in the population due to different seasons was also not significant (Table 84).

Actinomycetes population of the soil in different sets exhibited almost a similar trend. The population gradually increased in all the sets but more increase in the population was observed in the sets amended with the litter (Fig. 48-C). The peak population was observed in the month of September in unamended soil (UN), in January in the soil amended with fresh pine litter (Sl_1) and in October in the soil amended with semidecomposed litter (Sl_2).

Statistically no significant effect of litter amendments on actinomycetes population was observed. The variation in the population in different seasons was also not significant statistically (Table 85).

pH of the soil though varied significantly in different seasons, however, was not affected by pine litter amendment (Table 86).

2) Records of percentage relative abundance and frequency of fungal species in unamended and amended soil :-

Only seven fungal species, viz; 3 phycomycetes and four members of fungi imperfecti were isolated from the unamended soil whereas only six fungal species, viz; 2 phycomycetes and four members of fungi imperfecti were isolated from the soil amended with fresh leaf litter (Sl_1) 7 fungal species, viz; 2 phycomycetes and 5 members of fungi imperfecti were isolated from the soil amended with

semidecomposed litter (Sl_2) (Tables 87-89).

Absidia cylindrospora, Pythium sp and Trichoderma viride were found to be dominantly associated with all the three sets (Tables 87-89). The relative abundance of Absidia cylindrospora during different sampling periods was almost similar in all the three sets except in the month of September when it was found to be significantly low in the amended than in the unamended sets (UN), (Fig.50 and Tables 87-89). There was an increasing trend in the abundance of this fungal species from the month of July to September in soil amended with semidecomposed litter (Sl_2) and unamended sets but a decrease was recorded subsequently. Statistically no significant effect of litter amendment was observed on the abundance of this species in soil (Table 90).

The relative abundance of Pythium sp in soil during different sampling periods was almost similar in all the three sets. This species appeared in the soil with high relative abundance during the months of July and August. A decrease in the population was marked in the soil amended with fresh pine litter (Sl_1) from September to January. The population was almost same in the soil amended with semidecomposed litter (Sl_2) throughout the observation (Fig.50). There was no statistically significant effect of litter amendments on the abundance of this fungus in the soil, the variation which occured in the soil was due to the different seasons (Table 91).

The relative abundance of Trichoderma viride in

soil showed a somewhat different picture than the other two dominant species described above. The population was always higher in the soil amended with semidecomposed litter (Sl_2) but the population in the soil was not markedly affected with amendment of fresh pine needles (Fig.50). A decreasing trend in the population was marked in all the sets from the month of July to September and it increased subsequently from the month of October-January (Fig.50 and Tables 87-89). The variation in the percentage relative abundance of this species in soil was due to seasonal effect and was found to be statistically significant (Table 92).

It was interesting to note that though Penicillium chrysogenum was a occasionally occuring species of the unamended soil, the species appeared as a common form in the soil amended with fresh pine litter (Sl_1) and was almost not recorded from the soil amended with semidecomposed litter (Sl_2), (Fig.50 and Tables 87-89). The population of this species in soil of all the three sets was very low as compared to Absidia cylindrospora, Trichoderma viride and Pythium sp (Fig.50). Statistically no significant effect of pine litter amendments was observed on the abundance of this species in soil (Table 93).

A similar result was observed with Phoma humicola The fungus was isolated occasionally from the unamended soil and it appeared as a common species in the soil amended with semidecomposed litter (Sl_2) (Tables 87-89). The population was highest in the month of January in unamended soil and in soil amended with fresh pine litter (Sl_1) whereas it was

very low in the month of January in the soil amended with semidecomposed litter (Sl_2).

A species of Verticillium was recorded as rare species in all the three sets according to its percentage frequency (Tables 87-89).The population of this species, however, increased in the soil due to pine litter amendment (Fig.50).

3) Percentage germination of spores of certain soil fungi in pine litter leachates :-

The germination of spores of some dominant and common soil fungi in litter leachates collected after different periods of amendments was studied. Six fungal species namely, *Absidia cylindrospora*, *Trichoderma viride*, *Pythyium* sp, *Penicillium chrysogenum*, *Verticillium* sp and *Fusarium sporotrichoides* were selected for this study. Germination test was carried out in two different types of leachates collected from L_1 and L_2 litter during different periods.

The highest percentage of spore germination of all the test fungi was recorded in control sets (Fig.51). In general, percentage germination of all fungi in L_1 leachate at different periods of sampling was higher than in the leachates from L_2 litter (Fig.51). However, the germination of different fungi in leachates of L_1 litter was low in the samples collected in the months of July, August and September i.e. after 30,60 and 90 days of amendment, and it exhibited a gradual rise during the later part (Fig.51).

The inhibition in L_2 leachates was observed throughout the course of study for *Penicillium chrysogenum*

Fusarium sprotrichoides and *Verticillium* sp,. In case of *Absidia cylindrospora*, *Trichoderma viride* and *Pythium* species spore germination increased from the month of October to January (Fig.51).

Statistically significant effect of litter leachates on spore germination of the test fungi was recorded except for *Trichoderma viride* (Tables 95-100).

b) Percentage germination of fungal spores in soil washings of unamended and amended soil :-

Germination of spores of above mentioned fungi was also tested in the soil washings collected from unamended (UN)soil,soil amended with fresh pine litter (Sl_1) and soil amended with semidecomposed litter (Sl_2) during different sampling periods.

Spore germination of different fungi in unamended soil washings (UN) was stimulated almost throughout the course of investigation except in case of *Pythium* sp where inhibition was observed. The germination in the washings of soil amended with fresh pine litter (Sl_1) was also high throughout the course of investigation except from the month of October to January (Fig.52). A stimulatory effect on the germination of spores in the washings of soil amended with semidecomposed litter (Sl_2) was also observed in the samples collected during the months of July to September i.e. after 30, 60 and 90 days of litter amendment and thereafter the germination found to be inhibited (Fig.52).

The spore germination of *Penicillium chrysogenum*, *Verticillium* sp and *Fusarium sporotrichoides* in control

sets was higher than the unamended and amended sets (Fig.52).

Statistically no significant variation in the spore germination of Absidia cylindrospora, Pythium sp and Trichoderma viride was noticed between different sets of soil washings (Tables 101-103), but significant variation was observed for Penicillium chrysogenum, Verticillium and Fusarium sporotrichoides (Tables 104-106).

5) Detection of phytotoxins from decomposing pine litter and from unamended and amended soil :-

The liberation of phytotoxins from the decomposing L_1 and L_2 litter and from unamended and amended soil at different time periods was studied. It was observed that the phytotoxins were not liberated during months of September and October from the L_1 and L_2 litter. They could not also be detected in the soil during these months. The toxins were detected from the litter as well as from the unamended and amended soil samples in the months of July, August and January (Figs. 53-54).

On July caffeic acid was detected in L_1 litter leachates and Protocatechuic, Ferulic and Quinic acids were detected in L_2 leachates. Venillic acid was detected in unamended soil whereas protocatechuic acid was detected from soil amended with fresh pine litter (Sl_1) and semidecomposed litter (Sl_2) (Fig.53).

In the month of August 3,4 dihydroxy benzoic acid and Ferulic acid were detected in L_1 and L_2 litter leachates respectively. Gentisic acid was detected only from L_1 leachates (Fig.53).

Protocatechuic acid and 3,4 dihydroxy benzoic acid were detected in soil amended with fresh pine litter (Sl_1) and semidecomposed litter (Sl_2) respectively. Caffeic acid was detected in unamended soil (Fig.53).

In the month of January, protocatechuic acid and Caffeic acid were detected in L_1 and L_2 leachates respectively whereas 3,4 dihydroxybenzoic acid and protocatechuic acid were detected in unamended and amended soil (Fig.54).

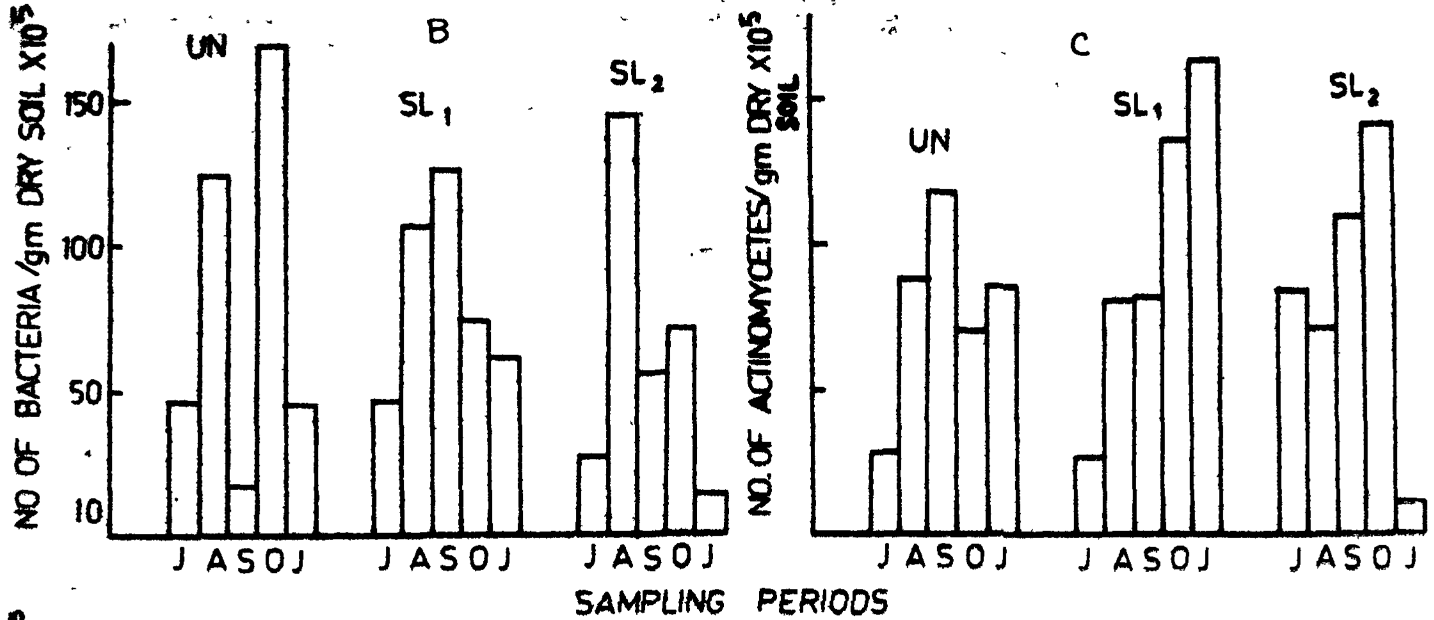
Figure - 48 : Showing the effect of amendment of pine leaf litter in the population of fungi, bacteria, actinomycetes in soil.
A, B, C.

Legends

UN = Unamended soil

SL_1 = Soil amended with fresh pine litter

SL_2 = Soil amended with semidecomposed litter.



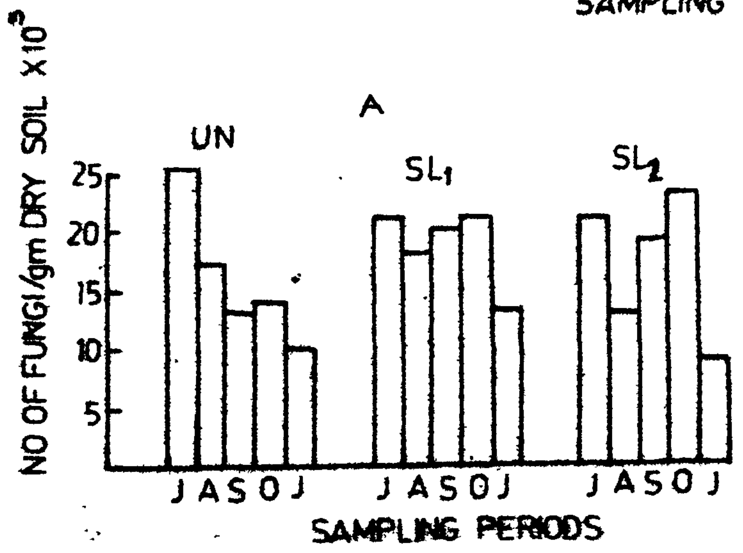


Fig. 48

Figure - 49 : Showing the effect of Pine leaf litter amendment on pH of the soil

<u>Legends</u> - UN = Unamended soil

SL_1= Soil amended with fresh pine litter

SL_2= Soil amended with semidecomposed litter.

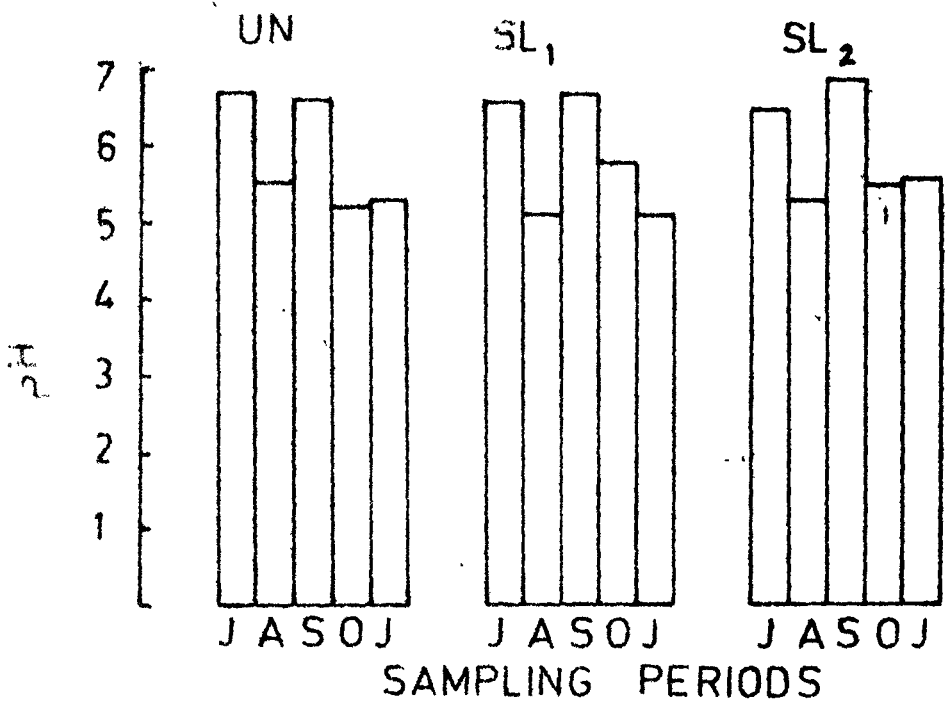


Figure 49

Figure - 50 : Histograms showing the percentage relative abundance of various fungal species isolated from the soil unamended (UN), and amended with fresh (SL_1) and semidecomposed pine litter (SL_2).

1 = *Absidia cylindrospora*, 2 = *Pythium* sp., 3 = *Trichoderma viride*, 4 = *Penicillium chrysogenum*, 5 = *Phoma humicola*, 6 = *Verticillium* sp.

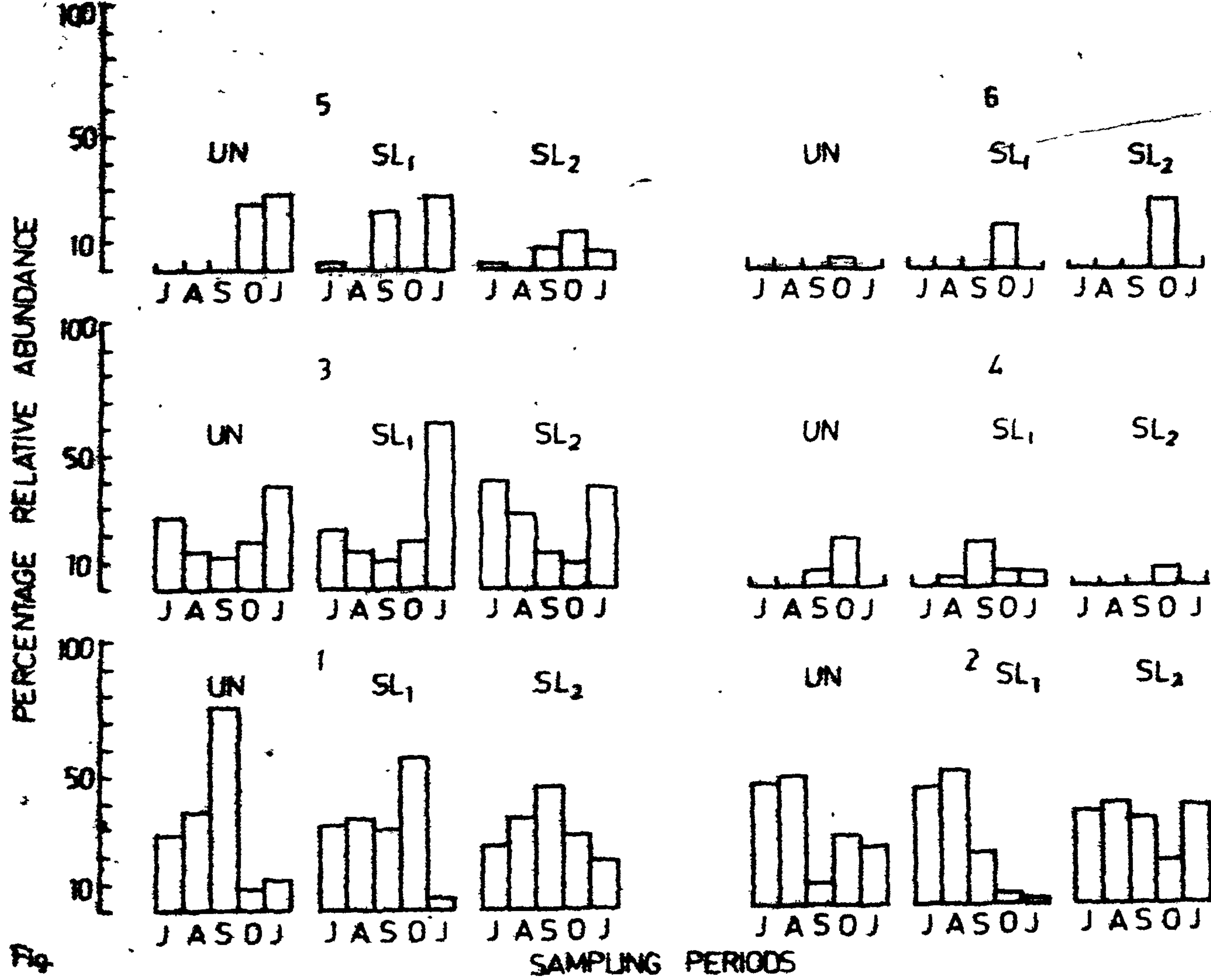


Figure 50

Figure - 51 : Effect of pine litter leachates on the spore germination of certain selected fungal species Leachates collected at different sampling periods. L_1 = Fresh pine litter, L_2 = Semidecomposed litter.

Selected fungi are :

1 = *Absidia cylindrospora*,
2 = *Pythium* sp., 3 = *Trichoderma viride*, 4 = *Penicillium chrysogenum*, 5 = *Verticillium* sp.,
6 = *Fusarium sporotrichoides*.

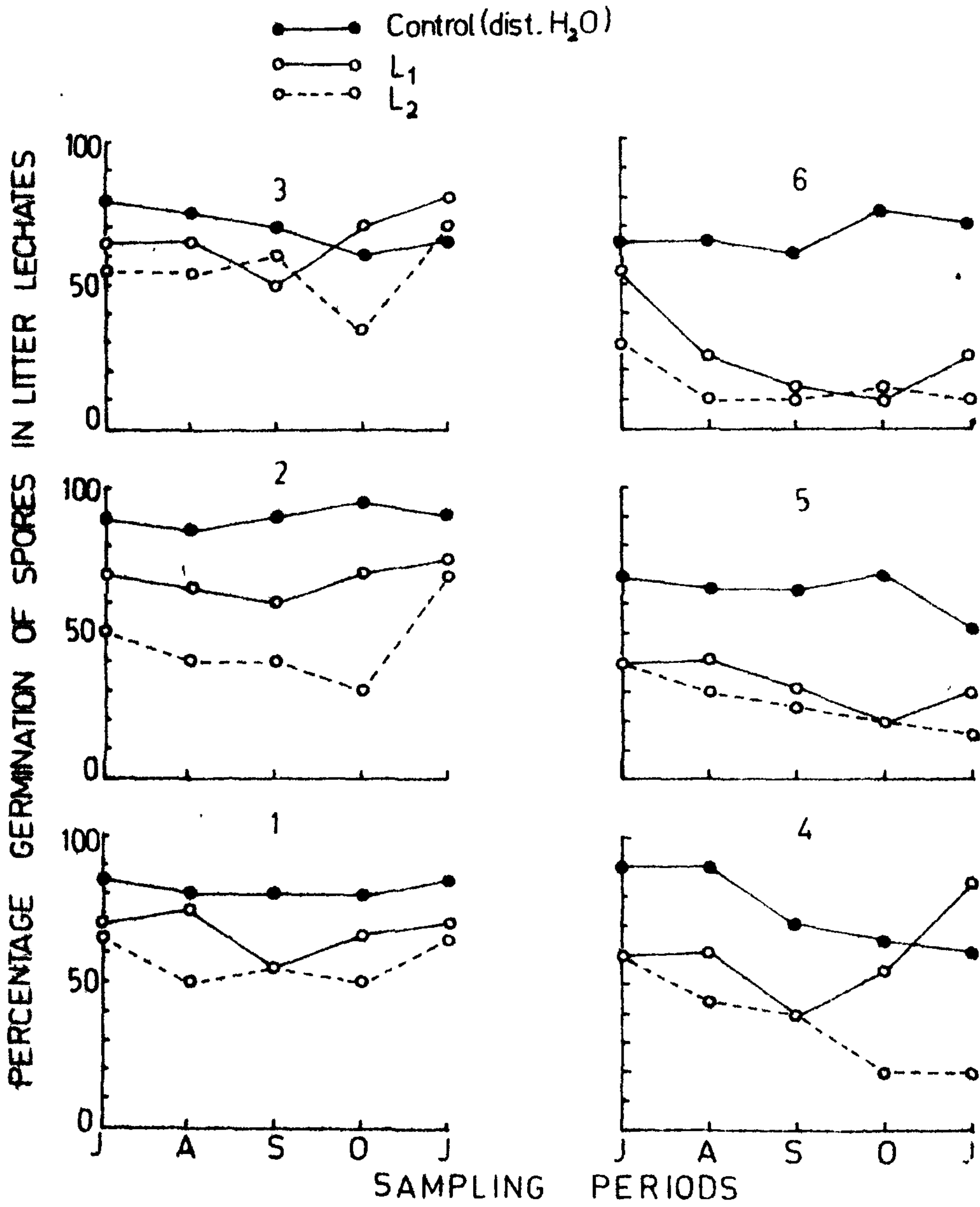


Figure 51

Figure - 52: Effect of different soil washings on the spore germination of certain fungal species.

UN = Washings collected from unamended soil

SL_1= Washings collected from soil amended with fresh pine litter.

SL_2= Washings collected from soil amended with semidecomposed litter.

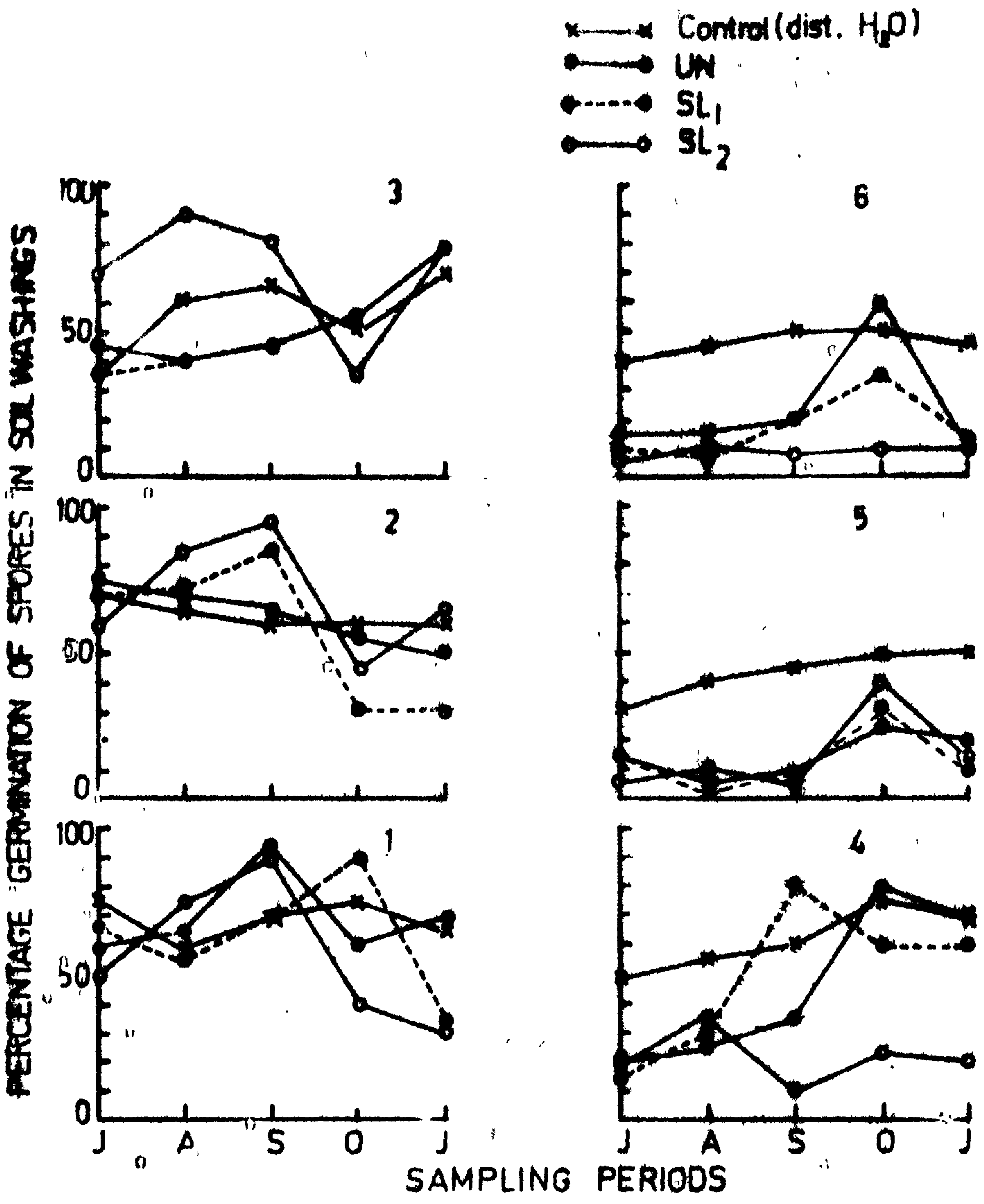


Figure 52

Figure - 53 : Chromatogram showing the presence of various phytotoxins in soil washings and litter leachates collected after various periods of amendments.

S(c) = Soil without litter

Sl_1 = Soil amended with fresh pine litter

Sl_2 = Soil amended with semidecomposed litter

L_1 = Fresh pine litter

L_2 = Semidecomposed litter.

Phytotoxins : 1 = 3,4 dihydroxybenzoic acid

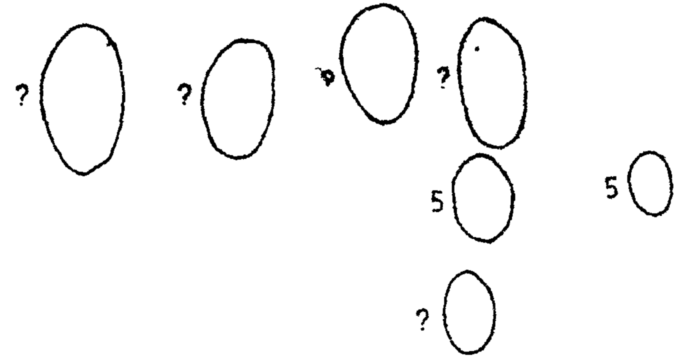
3 = Ferulic acid

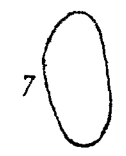
4 = Venillic acid, 5 = Quinic acid

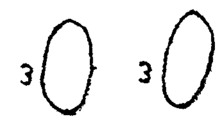
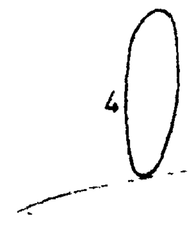
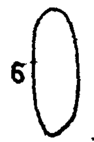
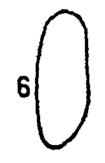
6 = Protocatechuic acid

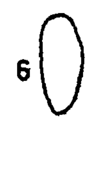
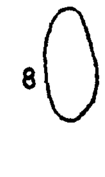
7 = Gentisik acid 8 = Caffeic acid

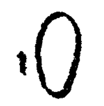
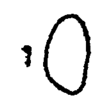
? = Unidentified spots.





Phytotoxins from soil amended with Pine leaf litter

Figure 53

Figure - 54 : Chromatogram showing the presence of various phytotoxins in soil washings and litter leachates collected after various periods of amendments.

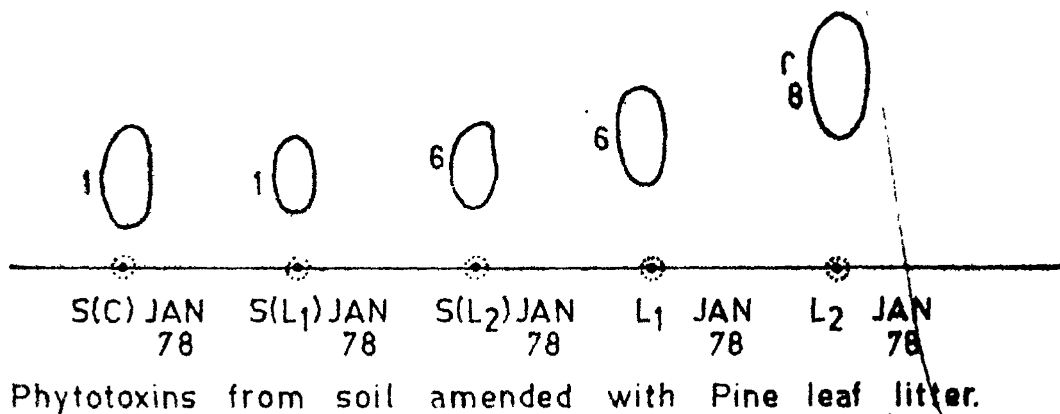
Phytotoxins :

1 = 3,4 dihydroxybenzoic acid

6 = Protocatechuic acid

8 = Caffeic acid

? = Unidentified spots.



Phytotoxins from soil amended with Pine leaf litter.

Figure 54

Table - 83. Analysis of variance for fungal population of soil unamended and amended with pine leaf litter.

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value		Remarks
					5%	1%	
Amendments	2	21.69	10.85	0.98	4.46	8.65	Not significant
Sampling periods (months)	4	217.50	54.38	4.93	3.84	7.01	Significant at 5% level of probabilities
Error	8	88.28	11.04				
Total	14						

The above table reveals that the fungal population of the soil did not vary significantly due to the pine litter amendment but it varies significantly due to the different seasons of the year.

Table - 84. Analysis of variance for Bacterial population of soil unamended and amended with pine leaf litter.

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value		Remarks
					5%	1%	
Amendments	2	1119.86	559.93	0.33	4.46	8.65	Not significant
Sampling periods (months)	4	17614.80	4403.70	2.60	3.84	7.01	Not significant
Error	8	13569.75	1696.22				
Total	14						

The above table reveals that the bacterial population of the soil neither varies significantly due to the pine litter amendment nor due to the different seasons of the year.

Table - 85. Analysis of variance for Actinomycetes population of soil unamended and amended with pine leaf litter.

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value		Remarks
					5%	1%	
Amendments	2	1059.83	529.92	0.01	4.46	8.65	Not significant
Sampling periods	4	8433.96	2108.49	0.02	3.84	7.01	Not significant
Error	8	750178.07	93772.26				
Total	14						

The above table reveals that the actinomycetes population of the soil neither varies significantly due to the pine litter amendment nor due to the different seasons of the year.

Table - 86. Analysis of variance for pH of soil (unamended and amended with pine leaf litter) during different sampling periods.

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value		Remarks
					5%	1%	
Amendments	2	0.03	0.02	0.40	4.46	8.65	Not significant
Sampling periods	4	6.09	1.52	30.40	3.84	7.01	Significant
Error	8	0.43	0.05				
Total	14						

The above table reveals that there is no statistically significant variation in pH of the soil due to the pine litter amendment but it varies significantly in different seasons of the year.

Table - 87. Percentage relative abundance and frequency of fungal species recorded in unamended soil in different samplings.

Species recorded	PERCENTAGE RELATIVE ABUNDANCE					Frequency
	JULY 77	AUG 77	SEP 77	OCT 77	JAN 78	
Absidia cylindrospora	28.0	37.0	76.0	9,0	13.0	D
Cunninghamella sp	1.0	-	-	-	-	R
Pythium sp	46.0	49.0	8.0	26.0	21.0	D
Phoma humicola	-	-	-	25.0	28.0	O
Trichoderma viride	25.0	14.0	12.0	19.0	38.0	D
Penicillium chrysogenum	-	-	4.0	18.0	-	O
Verticillium sp	-	-	-	3.0	-	R

Total spp = 7

D = Dominant (81-100% frequency); R = Rare (1-20% frequency);
O = Occasional (21-40% frequency).

Table - 88. Percentage relative abundance and frequency of fungal species recorded in SL_1 (soil amended with fresh pine leaf litter) in different samplings.

Species recorded	PERCENTAGE RELATIVE ABUNDANCE					Frequency
	JULY 77	AUG 77	SEP 77	OCT 77	JAN 78	
Absidia cylindrospora	32.0	33.0	30.0	57.0	4.0	D
Pythium sp	44.0	50.0	20.0	4.0	2.0	D
Phoma humicola	2.0	-	22.0	-	27.0	F
Trichoderma viride	22.0	24.0	11.0	18.0	62.0	D
Penicillium chrysogenum	-	3.0	17.0	5.0	5.0	C
Verticillium sp	-	-	-	16.0	-	R

Total spp = 6.

Table - 89. Percentage relative abundance and frequency of fungal species recorded in SL_2 (soil amended with semidecomposed pine leaf litter) in different samplings.

Species recorded	PERCENTAGE RELATIVE ABUNDANCE					Frequency
	JULY 77	AUG 77	SEP 77	OCT 77	JAN 78	
Absidia cylindrospora	29.0	34.0	46.0	28.0	18.0	D
Pythium sp	35.0	39.0	32.0	16.0	37.0	D
Phoma humicola	1.0	-	7.0	14.0	7.0	C
Geotrichum candidum	-	-	1.0	-	-	R
Trichoderma viride	40.0	28.0	14.0	10.0	38.0	D
Penicillium sp	-	-	-	6.0	-	R
Verticillium sp	-	-	-	26.0	-	R

Total spp = 7.

Table - 90. Analysis of variance for percentage relative abundance of Absidia cylindrospora in soil unamended and amended with pine leaf litter.

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value		Remarks
					5%	1%	
Amendments	2	19.60	9.80	0.03	4.46	8.65	Not significant
Sampling periods	4	2341.73	585.43	1.97	3.84	7.01	Not significant
Error	8	2383.07	297.88				
Total	14						

The above table reveals that the variation in the percentage relative abundance of *Absidia cylindrospora* in soil is neither due to pine litter amendments nor due to different seasons.

Table - 91. Analysis of variance for percentage relative abundance of *Pythium* sp in soil unamended and amended with pine leaf litter.

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value 5%	Table 'F' value 1%	Remarks
Amendments	2	166.80	83.40	0.60	4.46	8.65	Not significant
Sampling periods	4	2392.27	598.07	4.27	3.84	7.01	Significant at 5% level of probabilities
Error	8	1120.53	140.07				
Total	14						
£							

The above table reveals that the variation in the percentage relative abundance of *Pythium* sp in soil due to pine litter amendments is not significant, but it is significant at different seasons.

Table - 92. Analysis of variance for percentage relative abundance of *Trichoderma viride* in soil unamended and amended with pine leaf litter.

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value 5%	Table 'F' value 1%	Remarks
Amendments	2	56.93	28.47	0.33	4.46	8.65	Not significant
Sampling periods	4	2227.33	556.83	6.39	3.84	7.01	Significant at 5% level of probabilities
Error	8	697.07	87.13				
Total	14						

The above table reveals that the variation in the percentage relative abundance of *Trichoderma viride* in soil due to pine litter amendments is not significant, but it is significant at different seasons.

Table - 93. Analysis of variance for percentage relative abundance of *Penicillium* sp in soil unamended and amended with pine leaf litter.

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value		Remarks
					5%	1%	
Amendments	2	59.73	29.87	1.06	4.46	8.65	Not significant
Sampling periods	4	214.40	53.60	1.90	3.84	7.01	Not significant
Error	8	225.60	28.20				
Total	14						

The above table reveals that the variation in the percentage relative abundance of *Penicillium* sp in soil is neither due to pine litter amendments nor due to different seasons.

Table - 94. Analysis of variance for percentage relative abundance of *phoma* sp in soil unamended and amended with pine leaf litter.

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value		Remarks
					5%	1%	
Amendments	2	70.93	35.47	0.36	4.46	8.65	Not significant
Sampling periods	4	892.40	223.10	2.29	3.84	7.01	Not significant
Error	8	778.40	97.30				
Total	14						

The above table reveals that the variation in the percentage relative abundance of *Phoma* sp in soil is neither due to pine litter amendments nor due to different seasons.

Table - 95. Analysis of variance of germination of spores in Pine litter leachates.

Absidia cylindrospora

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value		Remarks
					5%	1%	
Between treatments	2	1580.14	790.07	26.64	4.46	8.65	Significant at 1% level of probability
Sampling periods	4	249.60	62.4	2.10	3.84	7.01	Not significant
Error	8	237.2	29.65				
Total	14						

The above table reveals that the variation in the germination of the spores of *Absidia cylindrospora* between litter leachates is statistically significant but there is no significant variation in germination of spores in the litter lechates collected at different seasons.

Table 96. Analysis of variance of germination of spores in pine litter leachates.

Pythium sp.

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value		Remarks
					5%	1%	
Between treatments	2	4840	2420	31.73	4.46	8.65	Significant at 1% level of probabilities
Sampling periods	4	490	122.5	1.60	3.84	7.01	Not significant
Error	8	610	76.25				
Total	14						

The above table reveals that the variation in the germination of the spores of Pythium sp between litter leachates is statistically significant but there is no significant variation in germination of spores in the litter leachates collected at different seasons.

Table - 97. Analysis of variance of germination of spores in pine litter leachates.

Trichoderma viride

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value		Remarks
					5%	1%	
Between treatments	2	603.4	301.7	2.745	4.46	8.65	Not significant
Sampling periods	4	490.06	122.5	1.114	3.84	7.01	Not significant
Error	8	879.94	109.9				
Total	14						

The above table reveals that the variation in the germination of the spores of *Trichoderma viride* between litter leachates and between different leachates collected at different seasons is statistically not significant.

Table - 98. Analysis of variance of germination of spores in pine litter leachates.

Penicillium chrysogenum

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value		Remarks
					5%	1%	
Between treatments	2	3709.8	1854.9	8.24	4.46	8.65	Significant at 5% level of probabilities
Sampling periods	4	1203.8	300.95	1.33	3.84	7.01	Not significant
Error	8	1800.2	225.02				
Total	14						

The above table reveals that the variation in the germination of the spores of *Penicillium chrysogenum* between litter leachates is statistically significant but there is no significant variation in germination of spores in the litter leachates collected at different seasons.

Table - 99. Analysis of variance of germination of spores in pine litter leachates.

Verticillium sp.

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value		Remarks
					5%	1%	
Between treatments	2	4199	2099.5	54.9	5.46	8.65	Significant at 1% level of probabilities
Sampling periods	4	590.3	147.5	3.86	3.84	7.01	Significant at 5% level of probabilities
Error	8	305.7	38.2				
Total	14						

The above table reveals that the variation in the germination of the spores of *Verticillium* sp between litter leachates and between leachates collected at different seasons is statistically significant.

Table - 100. Analysis of variance of germination of spores in pine litter leachates.

Fusarium sp.

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value		Remarks
					5%	1%	
Between treatments	2	7488.4	3744.2	19.74	5.46	8.65	Significant at 1 % level of probabilities
Sampling periods	4	758.6	189.65	1.85	3.84	7.01	Not significant
Error	8	819.0	102.37				
Total	14						

The above table reveals that the variation in the germination of the spores of *Fusarium* sp between litter leachates is statistically significant but there is no significant variation in germination of spores in the litter leachates collected at different seasons.

Table - 101. Analysis of variance for germination of spores in the extract of soil unamended and amended with pine litter. For Absidia cylindrospora.

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value		Remarks
					5%	1%	
Between treatments	3	543.75	181.25	0.693	3.49		Not significant
Sampling periods	4	1992.5	498.12	1.905	3.26		Not significant
Error	12	3137.5	261.45				
Total	19						

The above table reveals that the variation in the germination of the spores of Absidia cylindrospora between soil extracts is not statistically significant There is also no significant variation in germination of spores in the soil extract collected, at different seasons.

Table - 102. Analysis of variance for germination of spores in the extract of soil unamended soil and amended with pine litter.

For *Pythium* sp.

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value		Remarks
					5%	1%	
Between treatments	3	386.2	128.7	0.760	3.49		Not significant
Sampling periods	4	2734.7	683.6	4.040	3.26		Significant
Error	12	2031.3	169.2				
Total	19						

The above table reveals that there is a significant variation in the germination of pythium spores in the soil extract collected at different months

Table - 103. Analysis of variance for germination of spores in the extract of soil unamended and amended with pine litter.

Trichoderma viride

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value		Remarks
					5%	1%	
Between treatments	3	1240.6	413.5	2.09	3.49		Not significant
Sampling periods	4	2230.3	557.5	2.82	3.26		Not significant
Error	12	2364.3	197.0				
Total	19						

The above table reveals that the variation in the germination of the spores of *Trichoderma viride* between soil extracts is not statistically significant. There is no significant variation in germination of spores in the soil extract collected at different seasons.

Table - 104. Analysis of variance for germination of spores the extract of soil unamended and amended with pine litter.

Penicillium chrysogenum

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	'Table 'F' value' 5%	1%	Remarks
Between treatments	3	4265.35	1421.7	4.89	3.49		Significant
Sampling periods	4	2957.3	739.3	2.54	3.26		Not significant
Error	12	3483.9	290.3				
Total	19						

The above table reveals that the variation in the germination of the spores of *Penicillium chrysogenum* between soil extracts is statistically significant, but there is no significant variation in germination of spores in the soil extracts collected at different seasons.

Table - 105. Analysis if variance for germination of spores in the extract of soil unamended and amended with pine litter .

Verticillium sp

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value 5%	Table 'F' value 1%	Remarks
Between treatments	3	3013.75	1004.5	22.42	3.49		Significant
Sampling periods	4	1319.5	329.8	7.36	3.26		Significant
Error	12	538.5	44.8				
Total	19						

The above table reveals that the variation in the germination of the spores of *Verticillium* sp between soil extracts and between different extracts collected at different seasons is statistically significant.

Table - 106. Analysis of variance for germination of spores in the extract of soil unamended and amended with pine litter .

Fusarium sp

Sources of variations	D.F.	S.S.	M.S.S.	Variance ratio calculated	Table 'F' value		Remarks
					5%	1%	
Between treatments	3	3864.55	1288.1	14.78	3.49		Significant
Sampling periods	4	1220.3	305.0	3.50	3.26		Significant
Error	12	1045.7	87.14				
Total	19						

The above table reveals that the variation in the germination of the spores of *Fusarium* sp between soil extracts collected at different seasons is statistically significant.

DISCUSSION

Comparatively higher fungal and actinomycetes population was observed in amended soil (Figs.48 A, 48 B). This may be due to increased nutrients released from the amended materials particularly in the form of sugar and amino acids. Burges (1967) showed that the addition of plant residues to soil provides soluble and easily available energy materials which result in a "microbial explosion" of activity. High fungal counts in the soil of rich organic status has been reported by many workers (Saksena, 1955; Witkamp,1966; Dwivedi, 1966; Kamal 1968; Mishra, 1966; a,b; Mishra and Kanaujia 1972 C; and Kanaujia, 1973).

The low bacterial population in the amended soil even in the presence of favourable pH may be due to the antagonistic activity of actinomycetes population particularly Streptomyces sp in the soil (Figs.48,49).

Within the soil ecosystem relative efficiency of each group of microorganisms in terms of litter decomposition is determined by direct interactions between particular organisms. Many toxic or synergistic interactions may be considered in terms of the soil environment, on a bulk, micro or molecular scale. The organisms affect one another through changes brought about within these habitats. Such effects may be important in regulating the populations of organisms in soil.

A constant decrease in the fungal population in unamended soil (Fig.48 A) is perhaps due to the poor nutrient status and intercompetition between fungi for available food materials. There is evidence based on field

studies indicating that saprophytic activity in soil is governed by nutrient availability, particularly carbon and nitrogen (Forbes, 1974). More over presence of some phytotoxic compounds even in unamended soils suggests that these compounds are always present in the pine soil (Figs.53,54). Some of these compounds are highly volatile and perhaps inhibit the growth of fungi in unamended soil. Recent work on volatile components of plant residues in soil has shown that they play a part in inducing mycostasis (Balis et al, 1968; Hora et al, 1970).

Almost a constant fungal population in amended sets is primarily due to a constant supply of available nutrients from litter to underlying soil due to a gradual breakdown process (Fig.48 A).

The increase in the actinomycetes population in the amended sets almost throughout the observation is perhaps due to the ability of these group of organism to utilize more resistant organic residues such as cellulose and lignin of litter (Fig.48 C). These organisms are important in humifications, and they undoubtedly play an important role in gradual transformation of the organic amendments (Lu, 1968). Earlier workers have also reported the increase in actinomycetes when fresh organic matter is added to the soil, as at leaf fall or when crop residues of farm yard manure are ploughed in (Hiltner and Stormer, 1903; Jensen, 1931; Waksman et al 1939; Saito, 1966). However, they often colonize new substrates slower than bacteria and fungi.

The decrease in the fungi and bacteria at the end of the observation i.e. in the month of January, may be due

to the accumulation of more phytotoxic compounds in soil which affected adversely the growth of these organisms. The spore germination of certain soil fungi was inhibited in soil washings of both unamended and amended sets collected in January (Fig.52).

Almost no difference was observed in the qualitative nature of fungi in both unamended and amended soils (Tables 87-89). Absidia cylindrospora, Pythium sp and Trichoderma viride were dominant fungi irrespective of amendments (Fig.50). The pine litter amendment probably could not exert any significant effect on the soil microfungi. Abundance of these species irrespective of litter amendments may primarily be due to the presence of already available substrates in pine soil. Microhabitats of the soil may also play a decisive role for the growth of certain fungi in pine soil. However, the presence of certain phenolic compounds (phytotoxic substances) in the soil of both amended and unamended sets must have influenced the growth of Pythium, Absidia cylindrospora and Trichoderma viride. The germination of some common soil fungi in soil washings, collected from the both amended and unamended soil at the early stage of decomposition was stimulated (Fig.52). Menzies et al (1967) also observed an induced germination of fungi due to the presence of certain volatile components of plant residues in soil. Utilization of certain phenolic compounds by soil and litter fungi has already been confirmed by Black et al (1976).

Many phytotoxins were detected in the present study (Figs.53-54). They were generally observed during the months of July, August, and January. The production of phytotoxins

may be due to the active degradation of cellulose, hemicellulose, and lignin or the result of combined activity of decomposers. Takijima (1964) has found acetic, formic, butyric, lactic and succinic acids in rice paddy soils while Wang et al (1967) have reported phenolic acids sush as p-hydroxy benzoic acid, P-coumaric acid, Vanillic, ferulic and Syringic acids in sugarcane fields. These materials may affect soil microorganisms either by serving as food source or as growth inhibitors. The influence is felt, either directly or indirectly by the multitude of living components of that soil. It is believed that these toxic compounds are intermediate breakdown products of lignin, cellulose and other plant constituents (Patrick et al 1965).

GENERAL DISCUSSION

The decomposition of leaves is not a problem which is entirely confined to the litter on the forest floor. In fact, process of decay starts from the very moment the leaf is formed, and the leaves are exposed to attack by micro-organisms and animals during their whole life, senescence and death (Jensen, 1974). In this chapter, therefore emphasis will be given on the description of the whole series of events related to the succession of microorganisms specially fungi from the moment of unfolding of the pine needles until the decomposition of fallen litter. Also the various ecological factors that govern the decomposition of pine litter will be discussed.

It has been shown that tree leaves are invaded by microorganisms very rapidly after unfolding. Yeasts and filamentous fungi can also be demonstrated on the recently unfolded leaves. From the present investigation it has been demonstrated that fungi and yeasts were present on the pine needles even at bud stage. The population increased as the needles attained maturity (Fig.20). The source of infection presents no problem in ever green pine forest where microorganisms can easily be transferred from old to new leaves. Bacteria, yeasts and filamentous fungi have all been found in dormant buds of many trees (Davenport, 1966; Hislop and Cox, 1969; Pugh and Buckley, 1971a).

The phylloplane mycoflora of pine needles consisted of relatively few species, especially when the

needles were very young (Tables 47-49). This may probably be due to the exposure to the changing weather conditions, the limited avaliability of nutrients and the presence of antimicrobial substances (Topps and Wain, 1957; Beck et al 1969). As the needles grow, mature and senesce the activity of the fungi increases. With increase in the availability of nutrients the needles are invaded by several saprophytes and pathogens. Thus, at needle fall, a variety of fungi may be present in or on the needles (Miller, 1974).

From the present study an increase in number of species was recorded from the matured green needles until senescent stage. The highest number of species was recorded on litter, after leaf fall (Tables 50-52). During the bud stage of pine needles Aureobasidium pullulans and Sporobolomyces roseus were common and dominant species respectively (Table, 47). Aureobasidium pullulans as a most common filamentous fungus within dormant buds has also been reported by several earlier workers (Davenport, 1966; Pugh and Buckley 1971 b).As the needles grow older, other fungal species like Cladosporium herbarum, Penicillium chrysogenum, sterile white mycelia were more active and were abundant on the decomposing litter (Tables,48-52). Thus it seems that the fungi which were present on the decomposing litter were present from the unfolding of the needles.

The availability of different types of nutrients at different stages of plant growth is probably the main factor which determine the succession of microorganisms on the leaves. The population is affected by nutrient level of the leaf surface which changes with the release of the substances from the leaf.

Internal colonization of pine needles may be by parasitic and/or saprophytic organisms (Tables 53-58). Colonizers of latter group play a considerable part in decomposition of leaf tissue and parasitic forms often continue to function saprophytically after the death of the host material (Sadasivan, 1939; Gandy,1965,1966; Garrett,1970).

It is not possible to evaluate exactly the effects of the microbial activity on and in the leaves in the tree canopy. A certain proportion of the photosynthetic products formed by the leaves is, however, utilized by the microorganisms, and the decomposition of the leaves is already well under way when they reach the ground after leaf fall. It is also highly probable that leaf senescence is accelerated by microbial activity (Jensen, 1974).

The maximum number of fungal species was recorded on the decomposing litter (Tables 66-69). A wide range of deuteromycetes was recorded throughout the course of investigation and most of them were of common occurence. The deuteromycetes have been found to play the most important role in decomposition process. The dominance of other forms during various stages of decomposition may

be accounted to the selective response to substrate available at the time of decomposition (Mishra, 1971 a, b).

The occurence of *Pythium* sp. and *Mucor hiemalis* as dominant forms at the early stages of decomposition is due to their greater affinity towards sugar and amino acid (Figs. 25-27).

The presence of *Trichoderma viride*, *Penicillium chrysogenum* and *Cladosporium herbarum* on decomposing litter (Figs. 25-28) suggests their active role in decomposition of pine litter. These species are pulled up both from the newly fallen pine needles as well as from the debris already present on soil. The presence of *Trichoderma viride* and *Penicillium chrysogenum* in pine forest soil is well documented in the Chapter 1. Their role in litter decomposition under laboratory condition also suggests that they are the active decomposers of pine needles (Tables 37,38). Their wide occurence on decomposing litter could be explained in terms of their adaptation to utilize different phytotoxic compounds from decomposing litter (Black *et al*, 1976).

Most of the fungi recorded in the present study are so called 'soil fungi', but such a distinction between the two groups, viz. soil vs. leaf litter fungi is to a certain extent theoretical. Chesters (1949) and Pugh (1958) observed that many soil fungi are found in the leaf litter and the latter is possibly invaded first by the fungi growing on debris already present on the soil and the litter contributes available energy

materials to both the soil fungi and the leaf litter fungi. However, extremely common fungi like Trichoderma viride, Penicillium chrysogenum, Mucor hiemalis, Pythium sp. and many others which are encountered from the decomposing pine litter. This is probably due to their nutritional preferences.Among the phylloplane fungi remarkable reduction onthe decomposing needles was noted in the population of Aureobasidium pullulans and Sporobolomyces roseus (Tables 66-69). This suggests that they are less active on decomposing litter.

Surprisingly enough throughout the course of investigation the members of ascomycetes and basidiomycetes could not be observed in any appreaiable number. Some sterile white mycelia sometimes found with clamp connection are suspected to be basidiomycetous but in this case also it is with reservation only unless the fruiting bodies are observed.The role of the fungi of these two groups in decomposition of pine litter should not be underestimated because of their low occurrence. This may be due to the limitations of the techniques used and the media selected. Role of this group of the fungi on litter decomposition both in laboratory and field is worth investigating.

A pattern of succession of some common fungi on pine needles from bud to senescent to decomposing litter is given below as tabular form.

Buds	Unfolded needles
Sporobolomyces roseus	
Aureobasidium pullulans	--------
Penicillium chrysogenum	
Sterile white mycelia (Basidiomycetes)	--------

An overall picture of the study reveals that the distribution pattern of the fungal species on and within decomposing pine litter, besides other environmental factors, is regulated by the age of needles and the type of toxins present. The importance of the latter in pine litter decomposition is well decumented in the previous chapters.

Though the effort has been made in this study to investigate the different papameters related to pine litter decomposition under different ecological conditions, a number of question, however, still remains to be answered which will be looked intoin future studies.

Eg. a) The role of basidiomycetes in pine litter decomposition.

b) The role of mycorhizal fungi and their antagonistic effect against litter decomposing microbes.

c) Sterile mycelia and their role in litter decomposition.

d) Pattern of succession of bacterial and actinomycetes flora on pine litter and their role in decomposition.

The whole process of decomposition is, however, very complex. In field conditions particularly it is very difficult and unsafe to pin point the effect of individual factors on process of decomposition. Effort has been made to correlate the studies conducted in the field and laboratory conditions but within the limited period of investigation certain aspects could not be understood and they need further study.

ABSTRACT

Pinus kesiya Royle exGordon, has been selected for the present study beacuse of its wide distribution throughout this region and also because it produces more litter than do other tree species of the region. Five pine plantations varying from 9 years to 24 years age were selected for the present study.

The successive colonization of pine needles by fungi, bacteria and actinomycetes in relation to various environmental factors has been studied under different pine stands. To understand the biology of pine litter decomposition, soil microflora of pine forest was also assessed during the present investigation. The phylloplane fungi of pine needles were isolated simultaneously to assess their role in litter decomposition. The role of certain dominant fungi in pine litter decomposition was also estimated under laboratory condition. The role of pine litter amendment on soil microflora and the effect of litter leachates on the spore germination of certain fungi were investigated. The production of phytotoxins during pine litter decomposition was also analysed simultaneously.

Statistically no significant variation was observed in the fungal, bacterial and actinomycetes population of the soil of different pine plantations studied but a significant variation in the microflora was obtained during different seasons. During the soil microbial studies, relationships between Co_2 evolution, dehydrogenase activities, total microbial population and other physico chemical properties of soil was investigated

to assess the microbial activity of the forest soil. Statistically no strong correlation was observed between dehydrogenase activities and total microbial population of the forest soil. On the other hand a positive correlation was observed between Co_2 evolution and total microbial population of the soil. The addition of glucose as substrate substantially increased the dehydrogenase activities of the soil. A significant correlation was observed between dehydrogenase activities, soil moisture and pH of the forest. Soil moisture content did not effect the Co_2 evolution from the forest floor. Soil nitrogen content did not effect Co_2 evolution, dehydrogenase activities and total number of microbes at all. Extreme climatics fluctuations on the otherhand did exert a significant influence on the soil microbial population.

Regarding the qualitative nature of the soil mycoflora much difference was not marked between different plantations studied. Almost similar forms of fungi were recorded in all the plantations. *Absidia cylindrospora*, *Pythium* sp. and *Trichoderma viride* were the dominant soil fungi in all the plantations. The number of fungal species isolated from pine soil was not much. This is probably due to phytotoxic properties of the forest soil and also due to the presence of higher actinomycetes population which inhibited the growth of fungi. A remarkable feature of the forest soil was the absence of pathogenic fungi like *Fusarium* etc. There was a significant seasonal effect on the prevalence of different fungi in the soil.

As regard the phylloplane studies of pine needles

the fungal and yeast population of senescent needles was higher than the green needles. The population of the microfungi and yeast on the needles increased with the age of the needles and attained a peak when the needles enterted into the litter stage. Before the needles were subjected to decomposition they were inhabited by several leaf saprophytes. Statistically no significant variation was observed in the phylloplane fungal and yeast population of different plantations. It has also been observed that the microfungal and yeast population of the phylloplane of pine needles at different stages of maturity responded directly to the moisture content of the needles. Other environmental factors of this region also directly affected the fungal population of pine needles. Number of fungal species increased with the age of the needles and the highest number of species isolated was from the litter stage. Sporobolomyces roseus, Penicillium chrysogenum and a sterile white mycelia were dominant during the unfolded stage. When the needles attained maturity and entered into the senescent stage Cladosporium herbarum and sterile white mycelia were the dominant forms. Later on when the senescent needles entered into litter stage Sporobolomyces roseus and Cladosporium herbarum outnumbered sterile white mycelia and dominated the phylloplane of leaf litter.

During the field decomposition studies, fungal, bacterial and actinomycetes population of decomposing pine litter was minimum in the beginning and the population gradually increased with the advanced stage of decomposition. Moisture content of pine litter governed the variation in microbial population. Statistically a significant positive

correlation was obtained between moisture content and the microbial population of the decomposing litter. No significant variation in the microbial population of decomposing litter was obtained between different plantations. On the otherhand population was governed by the fluctuation inthe environmental conditions of the locality.

Qualitatively the number of fungal species isolated drom the decomposing litter was very low. Penicillium chrysogenum, Trichoderma viride and sterile white mycelia were the dominant fungi. The species of fungi imperfecti were over all dominant mycoflora during pine litter decomposition. The rate of breakdown of pine litter was positively correlated with evolution of Co_2. Also a positive correlation was obtained between the Co_2 evolution and total microbial population of decomposing litter. The changes in cellulose and hemicellulose content during litter decomposition was almost similar for all the stands. The loss of both the constituents followed total weight loss very closely. The lignin content of pine litter was degraded at extremely slow rate on the forest floor. The soluble components of the litter like total sugar and amino acid were lost very rapidly than any other components. In the laboratory decomposition studies the rate of pine litter decomposition was very high in the sets inoculated with Mucor hiemalis, Trichoderma viride, Cladosporium herbarum and mixed inoculum. On the otherhand, overall rate of decomposition was slower in the laboratory condition than under natural condition. This is accounted due tothe total exclusion of litter fauna in the experiment; Several

phytotoxins could be identified from the litter during various stages of decomposition under natural condition. Statistically no significant effect of litter amendment on fungal, bacterial and actinomycetes, population was observed under net house condition. The prevalence of certain fungal species in soil was, however, affected by pine litter amendment. Statistically, a significant effect of litter leachates on the spore germination of test fungi was also recorded. Fusarium sporotrichoides and Verticillium sp. were inhibited in the litter leachates throughout the course of germination test.

Lastly it can be concluded that among the fungi which were involved in pine litter decomposition, many of them were isolated from the phylloplane as well as from the forest soil of this region. The distribution of the fungi was largely affected by several biotic and abiotic factors.

BIBLIOGRAPHY

*Åbrahamsen, G. 1972. Pedobiologia 12 : 26-82.

Adams, P.S., Papavizas, G.C. and Lewis, J.A. 1968. Survival of root infecting fungi in soil III. the effect of cellulose amendment on chlamydospores germination of *Fusarium solani* f. sp. *phaseoli* in soil. Phytopathol, 58 : 373-385.

Alexander, M. 1961. In "Introduction to soil microbiology" John Wiley and sons, New York, pp.472.

Alexander, M. 1971. In "Microbial ecology" John Wiley and sons, New York.

Anderson, J.M. 1975. Succession, diversity and tropic relationships of some soil animals in decomposing leaf litter. J. Animal. Ecol. 44 : 475-495.

Ando, M. 1970. Litter fall and decomposition in some ever-green coniferous forests. Jpn. J. Ecol. 20 : 170-181.

*Bailey, J.A. 1969. Ann. Appl. Biol. 64 : 315-324.

Bailey, J.A. 1971. Phytoalexins and the ability of leaf tissue to inhibit fungal growth. In "Ecology of leaf surface microorganisms", pp. 519-528 (T.F. Preece and C.H. Dickinson, Eds.) Academic press, London.

Bainbridge, A. and Dickinson, C.H. 1972. Effect of fungicides on the mycoflora of potato leaves. Trans. Br. Mycol. Soc. 59 (1) : 31-41.

Baker, J.H. 1974. Comparison of the microbiology of four soils in Finish Lapland. Oikos, 25 : 209-215.

*Balis, C. and Kouyes, V. 1968. Annls. Inst. Phytopath, Benaki 9 : 145-149.

Barnett, H.L. and Hunter, B.B. 1972. In "Illustrated genera of imperfect fungi". Third Edition. Burges publishing company.

Barton, R. 1960. Saprophytic activity of Pythium mamillatum in soil. Trans. Br. Mycol. Soc. 43 : 529-540.

Bashi, E. and Fokkema, N.J. 1976. Scanning electron microscopy of Sporobolomyces roseus on wheat leaves. Trans. Br. Mycol. Soc. 67(3) : 500-505.

Bashi, E. and Fokkema, N.J. 1977. Environmental factors limiting growth of Sporobolomyces roseus, an antagonist of Cochliobolus sativus on wheat leaves. Trans. Br. Mycol. Soc. 68 (1) : 17-25.

Bauzon, D., Van Den Driessche, R. Dommergues, Y. 1968. Caracterization respirometrique at enzymatique des horizons de surface des sols forestiers. Science du sol No. 2 : 55-78.

*Bauzon, D. Van Den Driessche, R. and Dommergues, Y. 1969. L'effect litiére. I. Influence in situ des litiè res forestières sur quelques caractéristiques biologiques des sols. Cecol. plant. 4 : 99-122.

*Beck, C., Dommergues, Y. and Van Den Driessche, R. 1969. Oecol. pl., 4 : 237-266.

Bell, A. 1974. Fungal succession on dung of the Brush-tailed oppossum in New Zealand. New Zealand, Jr.Bot. 13 : 437-462.

Black, R.L.B. and Dix, N.J. 1976. Utilization of ferulic acid by microfungi from litter and soil. Trans. Br. Mycol. Soc. 66(2) : 313-317.

Black, R.L.B. and Dix, N.J. 1977. Colonization of scots pine litter by soil fungi. Trans. Br. Mycol. Soc. 68(2) : 284-287.

Blakeman, J.P. and Fraser, A.K. 1971. Inhibition of Botrytis cineria spores by bacteria on the surface of Chrysanthemum leaves. Physiological plant pathology. 1 : 45-54.

Blakeman, J.P. and Sztejnberg, A. 1973. Effects of surface wax on inhibition of germination of Botrytis cineria on beetroot leaves. Physiological plant pathology 3 : 269-278.

Bocock, K.L., Gilbert, O., Capstick, C.K., Twinn, D.C., Waid, J.S. and Woodman, M.J. 1960. Changes in leaf litter when placed on the surface of soils with contrasting humus type. I. losses in dry weight of Oak and Ash leaf litter. J. Soil. Sci. Vol.II, No.I, 2-9.

Borner, H. 1960. Liberation of organic substances from higher plants and their role in the soil sickness problem. Bot. Rev., 26 : 393-424.

Brandsberg, J.W. 1969. Fungi isolated from decomposing conifer litter. Mycologia 61 : 373-381.

Bray, J.R. and Gorham, E.1964. Litter production in forests of the world. Advances in ecological research 2 : 101-152.

Brian, A., Dickinson, C.H., and Goodfellow, M. 1977. Antagonistic interactions of phylloplane bacteria with Drechalera dictyoides. Can. J. Microbiol. 23 : 710-715.

*Broadfoot, W.M. and Pierre, W.H. 1939. Soil Science 48 : 329-348.

*Brown, W. 1922. Studies on physiology of parasitism. VIII. On the exosmosis of nutrient substances from the host tissue into the infection drop. Annals of Botany 36 : 101-119.

Bunnell, F.L., Tait, D.E.N., and Flanagan,P.W. 1977 . Microbial respiration and substrate weight loss II. A model of the influences of chemical composition. Soil. Biol. Biochem. 9 : 41-47.

Burges, A. 1939. Soil fungi and root infection. Broteria 8 : 35 Fasc. 2.

Burges, A. 1967. In "Soil biology" (A, Burges and F.Raw eds.), pp. 479-492. Academic press, London and New York.

Caldwell, R. 1963. Observations on the fungal flora of decomposing beech litter in soil. Trans. Br. Mycol. Soc. 46 : 249-261.

Carre, C.G. 1964. Fungal decomposition of beech cupules. Trans. Br. Mycol. Soc. 47 : 437-444.

Casida, L.E. Jr., Klein, D.A. and Santoro, T. 1964. Soil dehydrogenase activity, Soil. Sci. 98:371-376.

Chang, Y. and Hudson, J.H. 1967. The fungi of wheat straw compost. I. Ecological studies. Trans. Br. Mycol. Soc. 50 : 649-666.

Chavez, H.B., Bloss, H.E., Boyle, A.M. and Gries, U.A. 1976. Effect of crop residues in soil on Phytomatotrichum root rot of cotton. Mycopathologia, 58 : 1-8.

Chester, C.G.C. 1949. Concerning fungi inhabiting soil. Trans. Br. Mycol. Soc. 32 : 197-216.

Chester, C.G.C. 1950. On the succession of microfungi associated with the decay of logs and branches. Trans. Br. Mycol. Soc. Trans. Lines. Nat. Union., 12 : 129-135.

*Cholodney, N. 1932. Ber dt. bot. Ges. 50 : 562-570.

Christensen, M. 1960. The soil microfungi of conifer hardwood forests in Wisconsin. Diss. Abs. 20(1): 4253-4254.

Christensen, M., Whittingham, W.F. and Novak, R.O. 1962. The soil microfungi of wet mesic forests in Southern Wisconsin. Mycologia, 54 : 374-388.

Christensen, M. and Whittingham, W.F. 1965. The soil microfungi of open bogs and conifer swamps in Wisconsin. Mycologia, 57 : No.6 : 882-896.

Christensen, M. 1969. Soil microfungi of dry to mesic hardwood forests in Northern Wisconsin. Ecology, 50 : 9-27.

Collins, M.A. and Hayes, A.J. 1976. Seasonal incidence of microbes on the surface of first year needles of Norway spruce. Trans.Br.Mycol. Soc.66(3):457-461.

Crosse, J.R. 1971. Interactions between saprophytic and pathogenic bacteria in plant disease. In "Ecology of Leaf surface microorganisms" (T.F. Preece and C.H. Dickinson eds.) pp. 283-290. Academic Press. London.

Das, A.C. 1963. Ecology of soil fungi of rice fields. I. Succession of fungi on rice roots. Trans. Br. Mycol. Soc. 46 : 431-443.

Davenport, R.R. 1966. Report Long Ashton Research station for 1966, 246-258.

Davenport, R.R. 1967. Report Agricultural Horticultural Research station Univ. Bristol for 1966.

Davenport, R.R. 1968. Report Agricultural Horticultural Research station. Univ. Bristol for 1967.

Davenport, R.R. 1969. Report Agricultural Horticultural Research station. Univ. Bristol for 1968, 100-101.

Davey, C.B. and Papavizas, G.C. 1960. Effect of decomposing organic amendments and nitrogen on fungi in soil and bean rhizosphere. Trans. Intern. Cong. Soil. Sci., 7th.Cong. Medison, 2 : 551-557.

Davies, F.L. and Williams, S.T. 1970 Studies on the ecology of actinomycetes in soil. 1. the occurrence and distribution of actinomycetes in a pine forest soil. Soil. Biol. Biochem. 2 : 227-238.

*De Candolle, A.P. 1832. Excretion des racines. 3 : 1462-1492 (Becher Jeune, 1832).

Dickinson, C.H. 1965. The mycoflora associated with Helimione portulacoides III. Fungi on green and moribund leaves. Trans. Br. Mycol. Soc. 48(4) : 603-610.

Dickinson, C.H. and Morgan, J.G. 1966. The mycoflora associated with Helimione portulacoides IV. Trans. Br. Mycol. Soc. 49 : 43-45.

Dickinson, C.H. 1967. Fungal colonization of Pisum leaves. Can. J. Bot. 45 : 915-927.

Dickinson, C.H. 1971. Cultural studies of leaf saprophytes In "Ecology of leaf surface microorganisms" pp 129-137 (T.F. Preece and C.H. Dickinson eds.) Academic press, London.

Dickinson, C.H. 1976. Fungi on the aerial surfaces of higher plants. In "Microbiology of aerial plant surfaces". pp. 293-324 (C.H. Dickinson and T.F. Preece eds.) Academic press, London.

Dickinson, C.H. and Wallace, B. 1976. Effects of late application of foliar fungicides on activity of microorganisms on winter wheat flag leaves. Trans. Br. Mycol. Soc. 76 (1) : 103-112.

Dickinson, C.H. and O'Donnell, J. 1977. Behaviour of phylloplane fungi on Phaseolus leaves. Trans. Br. Mycol. Soc. 68 (2) : 193-199.

*di Menna, M.E. 1959. N.Z.J. agric. Res. 2 : 394-405.

di Menna, M.E. and Parle, J.N. 1970. Moulds on leaves of perennial ryegrass and white clover N.Z.J.agric. Res. 13 : 51-68.

di Menna, M.E. 1971. The mycoflora of leaves of pasture plants in NewZealand. In "Ecology of leaf surface microorganisms", pp 163 (T.F. Preece and C.H.Dickinson eds.) Academic press, London.

Dommergues, Y. 1964. Study of some factors influencing the behaviour of the soil microflora during the process of drying. Sci. Sol.141.

*Domsch, K.H. and Gams,W., Weber,E. 1968. Der Einfluss verschedenerVorfruchte auf des Bodenpilzspektrum in Weizenfoldern. Z. pflernahr. Bodenk. 119 : 134-149.

Domsch, K.H. and Gams,W. 1969. Variability and potential of a soil fungus population to decompose pectin, xylan and carboxymethyl-cellulose. Soil.Biol. Biochem. 1 : 29-36.

Douvigneaud, P. and Denaeyer-De smet, S.1970. Biological cycling of minerals in temperate deciduous forests. In "Ecological studies, analysis and synthesis". Vol.1. pp 199-225 (D.E. Reichle ed.) Springer verlag, New York.

Dransfield, M. and Mc donald, D.1966.The influence of cropping practices and application of farmyard manure on soil microflora at Samaru, Northern Nigeria. Nigeria Agric. J. 3: 42-55.

Drobnik, J. and Seifert, J. 1955. Beziehung der enzymatischen Inuession im Boden zu einigen mikrobiologis chen Testen. Folin Biol. Prague, 1 : 45-47.

Dwivedi, R.S. 1959. Soil fungi of grasslands of Varanasi. Ph.D. Thesis. B.H.U. (India). pp 119.

Dwivedi, R.S. 1966. Ecology of some soil fungi of some grasslands of Varanasi II. distribution of soil microflora. Trop. Ecol. 7 : 84-99.

Edwards, N.T. 1975. Effects of temperature and moisture on carbon dioxide evolution in a mixed deciduous forest floor. Soil science society of America proceeding. 39 : No.2, 361-365.

England, C.M. and Rice, E.L. 1957. A comparison of the soil fungi of a tall-grass prairie and of an abandoned field in Central Oklahoma. Bot. Gaz. 118 : 186-190.

Floate, M.J.S. 1970. Decomposition of organic materials from hill soils and pastures. Soil. Biol. Biochem. 2 : 187-196.

Fokkema, N.J. 1971. The effect of pollen in the phyllosphere of rye on colonization by saprophytic fungi and on infection by Helminthosporium sativum and other leaf pathogens. Netherlands journal of plant pathology 77.:Suppl. No.1, 1-60.

Fokkema, N.J. 1973. The role of saprophytic fungi in antagonism against Drechslera sorokiniana on agar plates and on rye leaves with pollen. Physiological plant pathol. 3 : 195-205.

Fokkema, N.J. 1976. Antagonism between fungal saprophytes and pathogens on aerial plant surfaces. In "Microbiology of aerial plant surfaces" pp 487-506, (C.H.Dickinson and T.F. Preece eds.). Academic press, London.

Fokkema, N.J. and Meulen, F.Van Der. 1976. Antagonism of yeast like phylloplane fungi against Septoria nodorum on wheat leaves. Netherlands. Journal of plant pathology. 82 : 13-16.

Forbes, R.S. 1974. Decomposition of agricultural crop debris. In "Biology of plant litter decomposition Vol.2" (C.H.Dickinson and G.J.F. Pugh eds.). pp 723-739. Acdemic press, London.

Foster, G.F. 1977. Effect of leaf surface wax on the deposition of airborn propagules. Trans. Br. Mycol. Soc. 68(2) : 245-250.

Frankland, J.C. 1966. Succession of fungi on decaying petioles of Pteridium aquilinum J. Ecol. 54 : 41-63.

Frankland, J.C. 1969. Funglal decomposition of bracken petioles. J.Ecol. 57 : 25-36.

Fraser, A.K. 1971. Growth restrictions of pathogenic fungi on the leaf surface. In "Ecology of leaf surface microorganisms" pp 529-535 (T.F.Preece and C.H. Dickinson eds.) Academic press, London.

Freddi, H. and Linderman, R.G. 1975. Effects of five acids that occur in pine needles on Fusarium chlamydospore germination in nonsterile soil phytopathology, 65 (10) : 1120-1124.

Gadgil, P.D. 1965. Distribution of fungi in living roots of certain gramineae and the effect of root decomposition and soil structure. plant and soil, 22 : 239-259.

*Galloway, L.D. 1936. Indian soil fungi. Indian J.Agric. Sci. 6 : 578-585.

Galstyan, A.S.H. 1964. Soil dehydrogenase. Dokl.Akad. Nauk, SSSR, 156 : 166-167.

Gandy, D.G. 1965. Itersonilia perplexans on chrysanthemums; some environmental factors affecting atmospheric spore concentration. Trans. Br.Mycol.Soc. 48 : 117-128.

Gandy, D.G.1966. Itersonilia perplexans on chrysanthemums; alternative hosts and ways of over wintering. Trans. Br. Mycol.Soc. 49: 499-507.

Garrett, S.D. 1951. Ecological group of fungi. A survey of substrate relationships. New phytol. 50 : 149-160.

Garrett, S.D. 1956. In "Biology of root infecting fungi" Cambridge Univ. press.

Garrett, S.D. 1963. In "Soil fungi and soil fertility" The Macmillan Company, NewYork.

Garrett, S.D. 1970. In "Pathogenic root infecting fungi" Cambridge Univ. press.

Garrett, H.E. and Cox,G.S. 1973. Carbondioxide evolution from the floor of an Oak-hickory forest Soil Sci. Soc. Amer. proc. 37 : 641-644.

Ghaffar, A., Zentmeyer, G.A. and Erwin, D.C. 1969. Effect of organic amendments on severity of Macrophomia root rot of cotton. Phytopathol; 59 : 1267-1269.

Ghosh, G.R. and Dutta, B.G. 1960. Soil fungi from Orissa (India). Mycologia 52 : 915-918.

Gilbert, O. and Bocock, K.L. 1960. Changes in the leaf

litter which placed on the surface of soils with contrasting humus types. II. changes in the nitrogen content of Oak and Ash leaf litter. Journal of soil science, vol.II. No.1, 10-19.

Gilman, J.C. 1956. In "A manual of soil fungi" (revised second eds.) Oxford and IBH publishing co.

Gilot, J.C. and Dommergues, Y. 1967. Note sur le lithosol calcaire a' more de la station subalpine de la R.C.P. 40. Rev. Ecol. Biol. Sol. 4 : 357-383.

Godfrey, B.E.S. 1974. Phylloplane mycoflora of bracken, Pteridium aquilinum. Trans. Br. Mycol. Soc. 62 : 305-311.

Goodfellow, M., Hill, I.R. and Gray, T.R.G. 1968. Bacteria in a pine forest soil. In "The ecology of soil bacteria an international symposium", pp 500-515 (T.R.G. Gray and D.Parkinson eds.) Liverpool Univ. press.

Gosz, J.R., Likens, G.E. and Borman,F.H. 1973. Nutrient release from decomposing leaf and branch litter in the Hubbard Brook forest, New Hampshire. Ecol. Monogr. 43 : 173-191.

Gray, T.R.G. 1969. Identification of soil bacteria. In "The soil ecosystem" (J.G.Sheels ed.) pp. 73-81 Systematics association, London.

Gray, T.R.G. and Williams, S.T. 1971. In "Soil micro-organisms" (T.R.G. Gray and S.T. Williams eds.) pp 99. Univ. Rev. Botany, Oliver and Boyd.

Gray, T.R.G. 1976. Survival of vegetative microbes in soil. In "The survival of vegetative microbes"

26th. Symposium of the society for general microbiology. pp 327-364, Cambridge Univ.press.

Gregory, P.H. and Hirst, J.M.1957. The summer air spores at Rothamsted in 1952. Journal of general microbiology 17 : 135-152.

Gregory, P.H., Lacey, M.E., Festenstein, G.N. and Skinner, F.A. 1963. Microbial and biochemical changes during the moulding of the hay. Journal of general microbiology. 33 : 147-174.

Gremmen, J. 1957. Microfungi decomposing organic remains of pines. Fungus 27 : 34-42.

Griffin, D.M. 1963. Soil moisture and ecology of soil fungi. Biol. Rev. 38 : 141-166.

Grover, S.K. 1977. Studies on fungal decomposition of Brassica nigra var. Sarson under different soil conditions. Ph.D. Thesis, Gorakhpur University (India).

Guenzi, W.L. and Mc Calla, T.M.1962. Inhibition of germination and seedling development by crop residues.Soil.Sci. Amer.proc. 26 : 456-458.

*Guillemat, J. and Montegut,J. 1957. Deuxime contribution a' letude de la microflora fongique des sols. cultives. Ann. Epiphyt. 8 : 185-207.

Guillemat, J. and Montegut, J. 1960. The effect of mineral fertilizers on some soil fungi.In "Ecology of soil fungi" pp 98-106 (D. Parkinson and J.S. Waid eds.) Liverpool Univ. press.

*Guittet, J. 1967. Oecol pl. 2 : 43-62.

*Hall, M.P. 1933. An analysis of the factors controlling the growth form of certain fungi, with special reference to _Sclerotinia_ (Monilia) _fractigena_ Ann. Bot. 47 : 538-578.

Hanis, V., Such, B. and Katan, J. 1967. The effect of organic amendments on _Rhizoctonia_ and accompanying microflora in soil. Can. J. Microbiol, 13 : 643-650.

Hathway, D.E. 1960. Plant phenols and tannins, In "_Chromatographic and electrophoretic technique_" pp 308-354, (J. Smith ed.) William Heinmann medical books ltd. London.

Hayes, A.J. 1965. Some microfungi from Scot pine litter Trans. Br. Mycol. Soc. 48 : 179-185.

*Heath, G.W. and King, H.G.C. 1964. Proc. VIII Intern. Congr. Soil. Sci. Bucharest 3 : 979-987.

Hering, E.P., 1965. Succession of fungi in the litter of a lake District Oakwood. Trans. Br. Mycol. Soc. 48 : 391-408.

*Hiltner, L. and Stormer, K. 1903. Studien uber die Bakerrienflora des Ackerbodens. Arb. K. Gesundh Amt. Biol. Abt. 3 : 443.

*Hiltner, L. 1904. Uber neure Erfrfahrungen und probleme auf dem Gebiet de Boden bacteriologie und unter besondere Beruck-schigung der Girundumngung under Brache. Arbdtdch, Landev, Ges. 98 : 59-78.

Hirte, W. 1963. Bemerkungen Zur Bestimung der Dehydrogenaseaktivitat im Boden. Zbl. Bakter. 116 : 478-484.

Hislop, E.C. and Cox, J.W. 1969. Effects of captan on the non parasitic microflora of apple leaves. Trans.

Br. Mycol. Soc. 52 : 223-235.

Hogg, B.M. and Hudson, H.J. 1966. Microfungi on leaves of *Fagus sylvatica*. 1.The microfungal succession Trans. Br. Mycol. Soc. 49 : 185-192.

*Holm, E. and Jensen,V. 1972. Cikos, 23 : 248-260.

Hora, T.S. and Baker, R. 1970. Nature, Lond. 225:1071-1072.

Howker, L.E. 1950. In "Physiology of fungi".University of London press Ltd., Bickley, Kent, England.

Huber, D.M. and Watson, R.D. 1970. Effect of organic amendments on soil borne plant pathogens. Phytopathology 60 : 22-26.

Hudson, H.J. and Webster, J. 1958. Succession of fungi on decaying stems of *Agropyron repens*. Trans. Br.Mycol. Soc. 41 : 165-177.

Hudson, H.J. 1968. The ecology of plant remains above the soil. New phytologist, 67 : 837-874.

Ivarson, K.C. and Katznelson,H. 1960. Studies on the rhizosphere microflora of yellow birch seedlings plant and soil XII. No.1. 30-40.

Jackson, M.L. 1958. In "Soil chemical analysis".

Jayasheela, N. and Oblisami, G. 1975. Plant microbe inter relationships in medicinal plants. 2.The rhizosphere microflora of *Withania sommifera.* South Ind. Horticulture. 23 : No.1 and 2, 7-12.

Jensen, H.L. 1931. The microbiology of farmyard manure decomposition in soil. I.Changes in the microflora and their relation to nitrification. J.Agric.Sci. 21 : 38.

Jensen, V. 1974. Decomposition of angiosperm tree leaf litter. In "Biology of plant litter decomposition" Vol.I. (C.H.Dickinson and G.J.F.Pugh eds.). Academic press, London.

Johri, B.N. and Thakre, R.P. 1975. Soil amendments and enrichment media in the ecology of thermophilic fungi. Proc. Ind. Nat. Sci. Acad. 41 : Part B, No.6, 564-570.

Jones, J.M. and Richards, B.N. 1977. Changes in the microbiology of Eucalypt forest soils following reafforestation with exotic pines. Aust. For. Res. 7 : 229-240.

Jung, G. 1970. Variations saisonnieres des caracteristiques microbiologiques dun sol ferru gineus tropical peu lessive (Dior), Soumis or non a l influence d Acacia albida (Del). Oecologia platarum 5 : 113-136.

Kamal, 1968. Ecological and taxonomical studies on soil fungi from teak forest of Gorakhpur, Ph.D. Thesis, University of Gorakhpur (India).

Kamal and Singh, C.S. 1979. Succession of fungi on decaying leaves of some pteridophytes. Ann. De L inst. pasteur, 119 : 468-482.

Kanaujia, R.S.1973. Studies on succession of fungi on root regio s of Pennisetum typhoides (Burm.F) Stapf and Hubb. Ph.D. Thesis, University of Gorakhpur (India).

Katznelson, B.H. and Stevenson, I.L.1956. Observations on metabolic activity of the soil microflora Can. J. Microbiology. 2 : 611-622.

Kendrick, W.B. 1957. Microfungi of pine litter. Ph.D. Thesis, University of Liverpool.

Kendrick, W.B. 1958. Microfungi of pine litter. Nature, (Lond.), 181 : 4322.

*Kendrick, W.B. 1959. Can. J. Bot. 37 : 907-912.

Kendrick, W.B. and Burges, A.1962. Biological aspects of the decay of Pinus sylvestris leaf litter. Nova Hedwigia 4 : 313-342.

Kerling, L.C.P. 1964. Fungi in the phyllosphere of leaves of rye and strawberry. Meded Landb. Hogesch. Opzoekstns Gent 29 : 885-895.

Khalifa, O. 1965. Biological control of Fusarium wilt of peas by organic amendments. Ann. Appl. Biol. 56 : 129-137.

Khanna, P.K. 1964. Succession of fungi of some decaying grasses. Ph.D. Thesis, B.H.U. (India).

Kiss, S.and Boaru,M. 1965. Methods for the determination of dehydrogenase activity in soil. In "Symposium on methods in soil biology" pp 137-143,Rumanian National society of soil science.

Kluyver, A. and Van Niel, C.B. 1956. "The microbes contribution to Biology". Harvard University press, Cambridge.

*Koef, H. 1954. Die biologische Aktivitat des Bodens undihre experimentelle Kennzichnung Z. pflanzenernahr Diing Bodenk. 64 : 138.

*Kornev, V.P. 1962. Pochvovedenie 11 : 109-114.

Kuster, R., Williams, S.T. 1964. Selectionof media for isolation of Streptomycetes. Nature, Lond. 202 : 928-929.

Lamb, D. 1976. Decomposition of organic matter on the forest floor of Pinus radiata plantations, J. Soil. Sci. 27 : 206-317.

Last, F.T.1955. Seasonal incidence of Sporobolomyces on cereal leaves. Trans.Br. Mycol. Soc. 38 : 221-239.

Last, F.T.and Deghton, F.C.1965. The non parasitic microflora on the surface of living leaves. Trans. Br. Mycol. Soc. 48 : 83-99.

Last, F.T.and Warren, R.C. 1972. Non parasitic microbes colonizing green leaves : their form and functions Endeavour, 31 : 143-150.

Latch, G.C.M. and McKenzie, E.H.C. 1977. Fungal flora of Ryegrass swards in Wales. Trnas. Br. Mycol. Soc. 68(2) : 181-184.

Leben, C. 1965. Epiphytic microorganisms in relation to plant disease. A. Rev. phytopath, 3 : 209-230.

Lenhard, G. 1956. Die Dehydrogenase aktivitat des Bodens als mass fiir die mikroorganismentatig keit im Boden. Z.pflanzenrnahr. Diing. Bodenk 73 : 1-11.

Lily, V.G. and Barnett, H.L. 1951. In "Physiology of fungi". Mc Graw Hill, Book Company, N.Y.

Lindsey, B.I. and Pugh, G.J.F. 1976a. A succession of microfungi on attached leaves of Hippophae rhamnoides. Trans. Br. Mycol.Soc. 67(1) : 61-67.

Lindsey, B.I. and Pugh, G.J.F. 1976. b. Distribution of microfungi over the surfaces of attached leaves of Hippophae rhamnoides. Trans. Br. Mycol. Soc. 67(3) : 427-433.

Lockwood, J.L. and Lingappa, B.T. 1963. Fungitoxicity of sterilized soil inoculated with soil microflora. phytopathol 53 : 917-920.

Lockwood, J.L. 1964. Soil fungistasis, Ann. Rev. phytopathol, 2 : 341-362.

*Lossaint, P. 1953. C.R. Acad. Sci, 236 : 522-524.

Lu, K.C. 1968. Effect of organic amendments on soil microflora in relation to Fusarium root rot of Ponderosa pine seedlings. Proc. West. Forest Conserv. Assoc. pp. 40-45.

Macauley, B.J. and Thrower, L.B. 1966. Succession of fungi in leaf litter of Eucalyptus regnans. Trans. Br. Mycol. Soc. 49 : 509-520.

Macauley, B.J. 1975. Biodegradation of litter in Eucalyptus pauciflora communities - 1. Technique. for comparing the effects of fungi and insects. Soil. Biol. Biochem. 7 : 341-344.

Mac Fadyen, A. 1970. Soil metabolism in relation to ecosystem energy flow and to primary and secondary production. In "Methods of study in soil ecology" (J. phillipson ed.). pp 167-172 IBP/UNESCO, Paris.

Mai, H. 1970. Bacterial succession in degradation of Alfalfa, rye straw and maize root. Zentr. Bakterial. II. 124 : 508-524.

Mallik, M.A. and Rice, E.L. 1966. Relation between soil fungi and seed plants in three successional forest communities in Oklahama, Bot. Gaz. 127 (2-3) : 120-127.

Mangenot, R. 1952. Researche methodiques sur les compignons de certain bais en decomposition. Rev. Gen. Bot. 59 : 381-399, 439-471, 477-519, 544-555.

Mangenot, F. 1966. Interactions nutritionnelles au niveau de la feville. Repport general. Annls. Inst. pasteur, Paris III (Suppl) : 329-341. ✓

Manning, W.J. and Crosson, D.E. 1969. Field and green house studies on the effect of the plant amendments on Rhizoctonia hypocotyl root of snap bean. plant Dis. Bull., 53 : 227-231.

Martin, P. 1957. Die Abgabe von organischen verbin dungen insbensen dire aus von scopoletin den kienwurzeln des Hafers (Avena saliva L) Arch Mikrobiol; 89 : 154-186.

Martin, J.P. 1950. Use of acid, rose bengal, and streptomycin in the plate method for estimating soil fungi. Soil Sci. 69 : 215-232.

Martin, J.P. and Juniper, B.E. 1970. In "The cuticle of plants" Edward Arnold,London.

Maurer, C.L. and Baker, R. 1964. Ecology of plant pathogens in soil I. Influence of chitin and lignin amendments on development of bean root rot. phytopathol; 54 : 1425-1426.

Maurer, C.L. and Baker, R. 1965. Ecology of plant pathogens in soil II. Influence of glucose, cellulose and inorganic nitrogen amendments on development of bean root rot. phytopathol; 55 : 69-72.

Mayer, F.H. 1960. Arch-Mikrobiol. 35 : 340-360.

Mayer, F.H. 1962. Z. pflernahr Dung. Bodenk. 97 : 234-243.

Mc Calla, T.M. and Duley, F.L. 1950. Stuble mulch studies III. Influence of soil microorganisms and crop residues on the germination, growth and direction of root growth of corn seedling. Soil. Sci. Soc. Amer. proc; 14 : 196-199.

Mckenzie, E.H.C. 1971. Seasonal changes in fungal spore numbers in rye grass-white clover pasture and the effects of benomyl on pasture fungi. New Zealand. J.Agri. Res. 14 : 379-392.

Mc kenzie,E.H.C. and Hudson, H.J. 1976. Mycoflora of rust infected and non-infected plant material during decay. Trans. Br. Mycol. Soc. 66(2) : 223-238.

Mc Laughlin, J.H. 1947. Isolation of Pythium from soil at various seasons of the year as related to the soil temperature and moisture phytopath. 37 : 15.

Mclean, H.C. and Wilson, G.W. 1914. Ammonification studies with soil fungi. N.J. Agric. Exp. station Bull. 270 : 1-30.

Mehrotra, R.S. and Tewari, D.P. 1976. Organic amendments and control of foot rot of piper betal caused by Phytophthora parasitica var. piperina. Ann. Microbiol. Inst. pasteur. A. 127 : 415-422.

Menzies, J.D. and Gilbert, R.G. 1967. Proc. Soil Sci. Soc. Amer. 31 : 495-496.

Menon, S.K. and Williams, L.E. 1957. Effect of crop, crop residues, temperature and moisture on soil fungi. phytopath 47 : 559-564.

Merril, W. and French, D.W. 1966. Colonization of wood by soil fungi. phytopathol; 56 : 301-303.

Metz, L.J.,1971. Pedobiologia II, 262-268.

Mikola, P. 1954. Experiments on the rate of decomposition of forest litter. Commun. Inst. For Fenn. 43 : 1-50.

Mikola, P. and Hintikka, V. 1956. The development of a microbiol population in decomposing forest litter publ. Forest. Rest. Inst. Finland 46 ; 1-15.

Mikola, P. and Hintikka,V. 1957. Commun. Inst.For. Fenn. 46 : 1-15.

Mikola, P. 1960. Comparative experiment on decomposition rates of forest litter in Southern and Northern Finland, Oikos, 11 : 160-166.

Miller, C.E. 1955. "Soil fertility". John Willey and sons, Ins. NewYork. Chapman and Hall, London, pp. 436.

Miller, C.E.; Turk, L.M. and Forth, H.D. 1958. "Fundamentals of soil science" John Willey and sons. Ins. NewYork Chapman and Hall. London pp.526.

Miller, C.S. 1974. Decomposition of coniferous leaf litter In "Biology of plant litter decomposition Vol-I" (C.H. Dickinson and G.J.F. Pugh eds.) pp 105-125. Acdemic press, London and NewYork.

Minderman, G. and Daniels, L. 1967. In "Progress in soil biology" (O.Graff and J.E. Satchell eds.) pp 3-9. North Holland, Amsterdam.

Mishra, R.R. 1964. Seasonal variation in fungal flora of grasslands of Varanasi. Ph.D. Thesis, B.H.U.(India) p 134.

Mishra, R.R. 1966.a. Influence of soil environment and surface vegetation on soil mycoflora. Proc. Nat. Acad. Sci. India, 26b. 117-123.

Mishra, R.R. 1966 b. Studies on the ecological factors governing the distribution of soil mycoflora. Proc. Nat. Acad. Sci. India 26 B : 205-222.

Mishra, R.R. 1967. Fungal population in relation to temperature and moisture. Proc. Nat. Acad. Sci. India 38 (B), III & IV.

Mishra, R.R. and Srivastava, V.B. 1970. Leaf surface microflora of Triticum sativum. Bull. Torr. Bot. Club. 97 (b) : 384-387.

Mishra, R.R. 1971 a. Effect of certain chemical fertilizers on the rhizosphere mycoflora of Oryza sativa I. Nitrogen fertilizers: Ammonium nitrate and urea. Mycopath et Mycol. Applic. 44:167-176.

Mishra, R.R. 1971 b. Effect of certain chemical fertilizers on the rhizosphere mycoflora of Oryza sativa Linn. II. Potassium fertilizers (pot. sulphate) Mycopath et Mycol. Applic. 45 : 119-124.

Mishra, R.R.and Srivastava,V.B. 1971 a. Leaf surface fungi of Oryza sativa Linn. Mycopath et Mycol Appl. 44 : 289-294.

Mishra, R.R. and Srivastava,V.B.1971b. Mycoflora of leaf surface in relation to virus infection. Proc. Nat. Acad. Sci. India 41(B) : 1, 90-92.

Mishra, R.R. and Srivastava, V.B. 1971 C. Aeromycology of Gorakhpur II. Spore content over a paddy field. Mycopath et Mycol Appl. 44 : 283-288.

Mishra, R.R. and Kanaujia, R.S. 1972. Studies on certain ecological aspects of soil fungi. Trop.Ecol. 13(1) : 5-11.

Mishra, R.R.and Kanaujia, R.S. 1973 a. Investigation into rhizosphere mycoflora XII.Seasonal variation in the mycoflora of certain gymnosperms. Sydowia, Annales Mycologici Ser II. Vol.XXVII, Heft 1-6, pp 302-311.

Mishra, R.R. and Kanaujia,R.S. 1973 b. Studies on certain ecological aspects of soil fungi II. Anales De Edafologia Y agrobiologia Tomo XXXII, Nums. 1-2, Madrid.

Mishra, R.R. and Tewari, R.P. 1976. Studies on biological control of Puccinia graminis tritici. In "Microbiology of aerial plant surfaces" (C.H.Dickinson and T.F. Preece eds.) pp 559-567. Academic press.

Mishra, R.R. and Sharma, G.D. 1977. Ecology of soil fungi : population variation in relation to varying cover vegetation and soil factors. Sydowia, Annales Mycologici Ser II. Vol XXX, heft 1-6, pp 134-140.

Mishustin, E.N., Pushkinskaya, O.I. and Teplyakova, Z.F. 1960. Ecologogeograficheskiye Zakonomernosti v rasprostraneii pochvenykh gribov. Izestiya Ann SSR Ser Biol. 5 : 641-665.

Mishustin, E.N. 1966. Geografichesky factor, pochvenyye tipy iikh mikrobroye naseleniye sbornik" Mikroflora pochv severnoi i Srednei chasti SSR Nauka, 3-23.

Mishustin, E.N., Mirzoyeva, V.A. 1968. Spore forming bacteria in the soils of USSR. In "The ecology of soil bacteria" pp 458-473. Liverpool University press.

*Morgan, J.V. and Tukey, H.B. jr. 1964. pl.physiol; Lancaster, 39 : 590-593.

Mortensen, U. and Staaf, H. 1975. Evolution of heat and carbondioxide from soil samples - a comparison of two methods for measuring the total activity of organisms in soil. Swedish coniferous forest project internal report 8 for 1975.

Muller, P.E. 1887. Studien uber die naturlichen Humusformen. Springer, Berlin.

Muller, K.O. and Borger, H. 1940. Arb. Biol. Rechsanstalt. Land. U. For stw., 23 : 189.

Nicot, M.M.E.J. 1960. Some characteristics of the microflora of desert sands. In "The Ecology of soil fungi". Liverpool Univ. press. pp 94-97.

Nielson, K.F., Cuddy, T.F. and Woods, W.B. 1960. The influence of extracts of some crops and soil residues on germination and growth. Can. J. plant Sci, 40 : 188-199.

*Nykvist, 1959. Oikos 10 : 212-224.

Okpala, E.V. 1975. Effect of organic soil amendments on 'damping off' of Lettuce caused by Corticum praticola Ann. Appl. Biol, 80 : 155-162.

Olson, J.S. 1963. Energy storage and the balance between producers and decomposers in ecological systems. Ecology 44 : 322-331.

Ordin, A.P. 1958. The influence of vegetation on the mycoflora of soils. Bull. Acad. Sci. U.S.S.R. 4 : 495-502.

*Paine, F.S. 1927. Studies on the fungus flora of virgin soil. Mycologia 19 : 248-267.

Park, D. 1954. Chlamydospores and survival insoil fungi Nature (Lond) 173 : 454-455.

Parkinson, D. and Kendrick, W.B. 1960. Investigations of soil microhabitats. In "Ecology soil fungi", pp 22-28, Liverpool Univ. press.

Parnas, H. 1975. Model for decomposition of organic material by microorganisms soil. Biol. Biochem. 7 : 161-169.

Patrick, Z.A. 1955. The peach replant problem in Ontario II. Toxin substances from microbial decomposition products of peach root residues. Can. J. Bot., 33 : 381-386.

Patrick, Z.A. and Koch, L.W. 1958. Inhibition of respiration, germiaation and growth by substances arising during the decomposition of certain plant residues in soil. Can. J. Bot. 36 : 621-647.

Patrick, Z.A. and Koch, L.W. 1963.The adverse influence of phytotoxic substances from decomposing plant residues on resistance of tobacco to black root rot. Can. J. Bot. 41 : 747-758.

*Patrick, Z.A., Toussoun, T.A. and Koch, L.W. 1964. Effect of crop residue decomposition products on plant roots. Ann. Rev. phytopath; 2 : 267-292.

Patrick, Z.A.and Toussoun, T.A. 1965. Plant residues and organic amendments in relation to biological control In "Ecology of soil borne plant pathogens" (Baker and Snyder eds.) pp. 440-459. University of California press, Berkley.

Patrick, Z.A. 1971. Phytotoxic substances associated with decomposition in soil of plant residues. Soil Sci. 3 : 13-18.

Peach, K. and Tracey, M.V. 1955. In "Modern methods of plant analysis". Springer verlag. Berlin, Germany.

Preece, T.F. and Dickinson, C.H. 1971. In "Ecology of leaf surface microorganisms" (T.F. Preece and C.H. Dickinson eds.) pp. 640. Academic press (Lond.).

Proebsting, E.L. 1950. A case history of peach replant situation. Proc. Amer. Soc. Hert. Sci., 56 : 46-48.

Pugh, G.F.F. 1958. Leaf litter fungi found on Carex panicullata L. Trans. Br. Mycol.Soc. 41 : 185-195.

Pugh, G.J.F. and Buckley, N.G. 1971 a. The leaf surface as a substrate for colonization by fungi. In "Ecology of leaf surface microorganisms" (T.F.Preece and C.H. Dickinson eds.) pp 431-445. Academic press, London.

Pugh, G.J.F. and Buckley, N.G. 1971 b. Trans. Br. Mycol. Soc. 51 : 227-231.

Pugh, G.J.F. and Mulder, J.L. 1971. Mycoflora associated with Typha latifolia. Trans. Br. Mycol. Soc. 57 : 273-282.

Pugh, G.J.F.and Lindsey,B.I. 1975. Studies on Sporobolomyces in a maritime habitat. Trans.Br. Mycol. Soc. 65 (2) : 201-209.

Rai, B. 1969. Saprophytic fungi on decaying leaves of Saccharum munja.Jr. Scientific. Res. B.H.U. Vol.XIX (2) : 34-39.

Rai, B. 1973. Succession of fungi on decaying leaves of Saccharum munja. Trop. Ecol. 14(2) : 102-128.

Rangaswami, G.; Oblisami, G. and Swaminathan, R. 1967. Antagonistic actinomycetes in the soils of South India.Univ. Agric. Sci.Bangalore and U.S.D.A.

Raper, K.B.;and Tham, C. 1949. Manual of the Penicillia. The Williams and Wilkins Co. Baltimore pp 875.

Reddy, G.S. and Rao, A.S. 1965. Effect of groundnut and Sesamum cakes on bacterial and fungal population of soil.Ind. J.Microbiol. 4 : 54-58.

Reddy, M.V.1979. Personal communication.

Reichle, D.E.; Dinger, B.E.; Edwards, N.T.; Harris, W.F. and Sollins, P. 1973. Carbon flow and storage in a forest ecosystem. In "proc. of the symposium carbon and Biosphere" (G.M. Woodwell and E.V. Pecan eds.) pp 392. NewYork.

Reiners, W.A. 1968. Carbondioxide evolution from the floor of three Minnesota forests. Ecol. 49 : 471-483.

*Remacle, J. 1970. Bull. Soc. R. Bot. Belg. 103 : 83-96.

*Remacle, J. 1971. Oikos 22 : 411-413,

Rentschler, I. 1971. planta, 96 : 119.

Rosswall, T.; Veum, A.K. and Karenlampi, L. 1975. Plant litter decomposition at Fennoscandian Tundra sites. In "Ecological studies and synthesis, vol.16, Fennoscandian Tundra ecosystems, part I" (Wielgolaski ed.) Springer-verlag, NewYork.

Ross, D.J. 1970. Effect of storage on dehydrogenase activities of soils. Soil. Biol. Biochem, 2 : 55-61.

Ross, D.J. and Roberts, H.S. 1970. Enzyme activities and oxygen uptakes of soils under pasture in temperatures and rainfall sequences. J. Soil. Sci. 21 : 368-381.

Ross, D.J. and Mc Neilly, B.A. 1972. Effects of storage on oxygen uptakes and dehydrogenase activities of beech forest litter and soil. N.Z. Journal of science 15 : 453-462.

Ross, D.J. and Mc Neilly, B.A. 1973. Biochemical activities in a soil profile under hard beech forest 3. Some factors influencing the activities of

polyphenol oxidising enzymes. N.Z. Journal of Science. 16 : 241-257.

Ruinen, J. 1961. An ecological neglected milleu plant and soil. 15 : 81-108.

Ruscoe, Q.W. Mycoflora of living and dead leaves of *Nothofagus truncata*. Trans. Br. Mycol. Soc. 56(3) : 463-474.
1971

Sadasivan, T.S.1939. Succession of fungi on decomposing wheat straw in different soils with specieal reference to *Fusarium culmorum*. Ann. Appl. Biol. 26 : 597-598.

*Saito,T.1956. Ecol.Rev. 14 : 141-147.

Saito, T. 1957. Chemical changes in beech litter under microbiological decomposition. Ecol.Rev.Tohoku, 14 : 209-16.

Saito, T.1960. Microbial decomposition of litter. Science. Reptr. Tohoku Univ. Ser 4, 26 : 125-131.

Saito, T. 1966. Sequential pattern of decompositionof beech litter with special reference to microbial succession.Ecol.Rev. 16 : 245.

Saksana, S.B. 1955. Ecological factors governing the distribution of soil microfungi inforests soils of Saugar. J.Ind. Bot.Soc. 63 : 339-357.

Schaefer, R. 1963. L' activite' des hydrogenasique comme mesure de l'activite' biologique globale des Sob. Ann. Inst.pasteur, 105, 326-31.

*Schoch, K. 1955. Ber. Schweiz.Bot.Ges. 65 : 205-50.

Seifert, J. 1965. The ecology of soil microbes Acta Univ. Carol. 3 : 245.

Sharma, P.D. 1967. Succession of fungi on decaying Setaria glauca Beauv. Ph.D. Thesis, B.H.U.India.

Singh, R.S. and Pandey, K.R. 1966. Effect of green and mature plant residues and compost on population of Pythium aphanidermatum in soil.Ind.phytopath, 19 : 367-372.

Singh, J. 1937. Soil fungi and actinomycetes in relation to manural treatment, season and crop. Ann. appl. Biol., 24 : 154-168.

Singh, P.N. 1967. Comparative study of the fungal population in the rhizosphere of Forbs growing under natural and artificial conditions. Ph.D. Thesis. B.H.U. India.

Sinha, S.1965. Microbiological complex of the phyllosphere and disease control.Ind. phytopath. 18 : 1-20.

Sinha, S. 1971. The mycoflora on leaves of Capsicum annum (L),Solanum melongena(L), Solanum tuberosum (L), and Lycopersicum esculantum Mill. In "Ecology of leaf surface microorganisms" (T.F.Preece and C.H. Dickinson eds.) pp 175-189.

Siu, R.G.H. and Reese, E.T. 1953. Decomposition of cellulose by microorganisms. Bot. Rev. 19 : 377-416.

*Skinner, J.J. 1918. Soil aldehydes, a scientific study of a new class of soil constituents unfavourable to crops, their occurrence properties and estimation in practical agriculture. J. Franklin. Inst. 180 : 165-168.

Skujins, J. and Mclaren, A.D. 1968. Persistance of enzymatic activities in stored and geologically preserved soils. Enzymologia, 34 : 213-225.

Smith, R.S.Jr. 1967. Decline of Fusarium oxysporum in the root of Pinus lambertiana seedlings transplanted into forest soils. Phytopath 57 : 1265.

Smith, J. and Wieringa,K.T. 1953. Microbial decomposition of litter. Nature (Lond.) 171 : 794-795.

Snyder, W.C., Scroth,M.N. and Christon,T. 1959. Phytopathology 49 : 755-756.

Snyder, W.C. 1960. Antagonism as a plant disease control principles. In "Biological and chemical control of plant and animals pests". Amer. Assoc. for the advancement of science. pp 127$136.

*Sokolov, D.F. and Karpova, N.V. 1965. Lesa podmoskovya, 63-99.

Sondheimer, E. 1961. Possible identity of a fungitoxic compound from carrot root. Phytopathol 51 : 71-72.

Srivastava, V.B. and Mishra, R.R. 1971. Investigations into rhizosphere microflora III. Influence of sampling time. Microbiologia. Espanola, 24 : 115.

Stevenson, I.L. 1959. Dehydrogenase activity in soils. Can. J. Microbiol. 5 : 229-235.

Stotzky, G. 1965. Microbial respiration. In "Methods of soil analysis II. Chemical and microbiological properties" (Black, C.A. edn) pp 1550-1570. Amer. Soc.Agronomy, Medison.

Stotzky, G. and Rem, L.T.1966. Influence of clay minerals on microorganisms. 1. Montmorillonite and kaolinite on bacteria.Can J. Microbiol, 12 : 547.

Stotzky, G. 1974. Activity, ecology and population dynamics of microorganisms in soil. In "Microbial ecology" (Laskin,A.I. and Lechevalier, H. eds.) CRC press,Cleveland, pp 57.

*Stout,J.D. 1961. N.Z. J. Agric. Res. 4 : 1-30.

Stover, R.H. 1962.Fusarial wilt (Panama disease) of banana and other Musa species. Commonwealth mycological. Institute (Kew England), Phytopathol paper No.4 : 117.

Subramanian, C.V. 1971. In "Hypomycetes an account of Indian species", except Cercospora. ICAR publications.

Takijima, Y. 1964. Studies on organic acids in paddy field soils with reference to their inhibitory effects on the growth of rice plants.Soil Sci. plant. nutr. 10 : 14-29.

Tanaka,H.and Sakamato,M. 1972. Studies on soil physical conditions and the ecology of soil borne disease bacteria. 4. the relationships between bacterial numbers and either soil water potential (pf) or moisture contents. Bull. Inst. Agric. Res. Tohoku Univ., 23 : 141.

Teplyakova, Z.F.1961. Aktinomitsety gornykh i podgornykh pochv zaitiyskogo Ala-Tau. Sbornik "Fiziologiya i Ekologiya Mikroorganizmov". Publ. n. An.

Kaz SSR, p. 129-138.

Thalmann, A. 1968. Zur Methodik der Bestimmung der Dehydrogenaseaktivitat im Boden mittels Triphenyltetrazolium chloride (TTC).Landw. Forsch. 21 : 249-258.

*Topps, J.H.and Wain,R.L. 1957. Nature (Lond.).179 : 652-653.

Toussoun,T.A.and Patrick,Z.A.1963. Effect of phytotoxic substances from decomposing plant residues on root rot of bean. Phytopathol, 53 : 265-270.

Toussoun, T.A., Weinhold, A.R,Linderman,R.G. and Patrick, Z.A. 1968. Nature of phytotoxic substances produced during plant residue decomposition in soil phytopathol, 58 : 41-45.

Toussoun,T.A., Menzinger,W. and Smith,R.S. Jr. 1969. Role of conifer litter in ecology of Fusarium. Stimulation of germination in soil phytopathol, 59 : 1396-1399.

Tresner, H.D., Boskus,M.P. and Curtis,J.T.1954. Soil microfungi in relation to the hardwood forest continum in Southern Wisconsin, Mycologia 46 : 314-33.

Tribe,H.T. 1957.Ecology of microorganisms in soils as observed during their development uppon burried cellulose film. Symp. Soc. Gen. Microbiol; 7 : 287-298.

Tribe, H.T. 1960 a. Aspects of decomposition of cellulose in Canadian soils. I. Observations with microscope. Can. J. Microbiol. 6 : 309-316.

Tribe, H.T. 1961. Microbiology of cellulose decomposition in soil. Soil. Sci. 92 : 61-77.

Tubaki,K. and Saito,T. 1969. Endophragmia alternata Sp Nov. and other hypomycetes on Pinus leaves in Japan. Trans. Br. Mycol. Soc. 52 : 477-482.

Tubaki,K. and Yokoyama,T. 1971.Successive fungal flora on sterilized leaves in the litter of forests 1. I.F.O. Res. Comm. 5 : 24-42.

*Tukey,H.B., Jr. and Morgan,J.V. 1963. Physiologia pl. 16 : 557-565.

Tukey, H.B. 1971. In "Ecology of leaf surface micro-organisms" (T.F.Preece and C.H.Dickinson ed) pp. 67-80, Academic press, London.

Tyner, L.E. 1961. Colonisation of organic matter in soil by fungi. Phytopathol, 51 : 625-634.

*Usher,M.B. 1970. Pedobiologia, 10 : 224-236.

Vaartaja, O. 1975. Pythium sylveticum in canadian forest nurseries. Can. Plant. disease survey. 55:101-102.

Verstraete,W. and Voets,J.P. 1976. Soil microbial and biochemical characteristics in relation to soil management and fertility.Soil. Biol. Biochem. 9 : 253-258.

Vishwanath,D., Patil,R.B.and Rangaswamy,G. 1975. Dehydrogenase activity and microbial population in amended and unamended soils with incubation.ZBl. Bakt. Abt. II, B'd. 130 : 123-130.

Vittal,B.P.R. 1976. Studies on litter fungi 1. Mycoflora of Atlantia and Gymnosporia litter : Proc. Indian. Acad. Sci., 83B (4) : 133-138.

Vladimir, T. 1970. Decomposition of Ligno cellulose during successive action of the fungi Ganoderma applanatum and Serpula lacrymans. Folia. Tomus XI, Biologia 27 : Opus 6.

Waid, J.S. 1957. Distribution of fungi within the decomposing tissues of rye grass roots. Trans. Br. Mycol. Soc. 40 : 391-406.

Waksman, S.A. 1917. Is there any fungus flora of the soil? Soil. Sci. 3 : 565-589.

Waksman, S.A. and Starkey, R.L. 1924. Influence of organic matter upon development of fungi, actinomycetes and bacteria in soil. Soil. Sci. 12 : 373-378.

Waksman, S.A.; Umbreit, W.W. and Cordon, T.C. 1939. Thermophilic actinomycetes and fungi in soils and in composts. Soil. Sci. 47 : 37.

Waksman, S.A. 1952. In "Soil microbiology" John Wiley and sons, N.Y.

*Walkley and Black. 1934. Soil. Sci. 37 : 29.

Walter, H. and Haber, W. 1957. Ueber die intensitat der Bodenstmung mit Bemerkungen zu den Lundegardhschen Werten. Ber. dtsch. bot. Ges. 70 : 275-282.

Wang, T.S.C., Yang, T.K. and Chang, T.T. 1967. Soil phenolic acids as plant growth inhibitors. Soil. Sci. 103 : 239-246.

Warcup, J.H. 1950. The soil plate method for isolation of fungi from soil. Nature (Lond.) 166 : 117.

Warcup, J.H. 1951. The ecology of soil fungi. Trans. Br. Mycol. Soc. 34 : 379-399.

Warcup, J.H. 1957. Studies on the occurrence and activity of fungi in a wheat field soil. Trans. Br. Mycol. Soc. 40 : (2), 237-263.

Ward, G.M. 1952. Studies in the succession of fungi in decomposing litter of coniferous forest soils. Ph.D. Thesis, University of Nottingham.

*Wardlaw, I.F. 1968. Bot. Rev. 34 : 79-105.

Warren, R.C. 1972.a. The effect of pollen on the fungal leaf microflora of *Beta vulgaris* L. and on infection of leaves by *Phoma betae*. N.Z. plant Pathol. 78 : 89-98.

Warren, R.C. 1972.b. Attempts to define and mimic the effects of pollen on the development of lesions caused by *Phoma betae* inoculated on to sugarbeet leaves. Ann. Appl. Biol. 71 : 193-200.

Warren, R.C. 1976. On manipulating fungal microfloras on leaves. Trans. Br. Mycol. Soc. 67(1).

Watson, E.S.; Mc Clurkin, D.C. and Huneycutt, M.B. 1974. Fungal succession on Loblolly pine and upland hardwood foliage and litter in North Mississippi Ecology 55(5) : 1128-1134.

Webster, J. 1956. Succession of fungi on decaying cockfoot culms. I. J. Ecol. 44 : 517-544.

Webster, J. 1957. Succession of fungi on decaying cock foot culms. II. J. Ecol. 45 : 1-30.

Wicklow, T.D. and Whittingham, W.F. 1974. Soil micro-fungal changes among the profiles of disturbed conifer - Hardwood forests. Ecology 55 : 3-16.

Widden, P and Parkinson, D. 1973. Fungi from Canadian coniferous forest soils. Can. J. Bot., 51(12): 2275-2290.

Williams, S.T. 1963. The distribution of fungi in the horizons of a podsolized soil. In "<u>Soil organisms</u>" pp 158-166. Amsterdam. North Holland publishing co.

Williams, S.T., Davies,F.L. 1965. Use of antibiotics for selective isolation and enumeration of actinomycetes in soil. J. gen. Microbiol. <u>38</u>:251-261.

Williams, S.T. and Mayfield,C.I. 1971. Studies on the ecology of actinomycetes in soil III. the behaviour of neutrophilic <u>Streptomycetes</u> in acid soil. Soil. Biol. Biochem. <u>3</u> : 197.

Williams, S.T.; Davies,F.L.; Mayfield,C.I. and Khan,M.R, 1971. Studies on the ecology of actinomycetes in soil II. The pH requirements of <u>Streptomycetes</u> from two acid soils. Soil. Biol. Biochem. <u>3</u> : 187-195.

Williams,S.T. and Gray,T.R.G. 1974. Decomposition of litter on soil surface. In "Biology of plant litter decomposition Vol.II" (C.H.Dickinson and G.J.F. Pugh eds.) pp 615-619.

Wingfield, G.I.; Davies,H.A. and Greaves,...P. 1977. The effect of soil treatment on the response of the soil microflora to the herbicide Dalapon. J. applied. Bacteriology, <u>43</u> : 39-46.

Witkamp, M. 1963. Microbial populations of leaf litter in relation to environmental conditions and decomposition. Ecology <u>44</u> : 370-377.

Witkamp, M. 1966. Decomposition of leaf litter in relation to environment, microflora, and microbial respiration. Ecology <u>47</u> : 194-201.

Witkamp, M. 1971. Forest soil microflora and mineral cycling In "Productivity of forest ecosystems" (P. Duvigneand ed.) pp. 413-424. UNESCO,Paris.

Woodwell, G.M. and Botkin,B.B. 1970. Metabolism of terrestrial ecosystems by gas exchange techniques in "Analysis of temperate forest ecosystems" (D.E. Reichle ed.) pp 304. Springer-verlag, NewYork.

Wright, E. and Bollen, W.B. 1961. Microflora of Douglass-fir forest soil. Ecol. 42(4) : 825-828.

Yadava, A.S. 1966. The ecology of microfungi on decaying stems of *Heracleum sphondylium* L. Trans. Br. Mycol.Soc. 49 : 47-86.

---

* Original not seen.

NEHU Library
A... 58352